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(54) **ELECTROSPRAYING METHOD WITH CONDUCTIVITY CONTROL**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 868 days.

This patent is subject to a terminal disclaimer.

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US 2008/0141936 A1 Jun. 19, 2008

Related U.S. Application Data

(60) Continuation of application No. 10/809,660, filed on Mar. 25, 2004, now Pat. No. 7,279,322, which is a division of application No. 10/162,031, filed on Jun. 3, 2002, now Pat. No. 6,746,869, which is a continuation of application No. 09/577,747, filed on May 23, 2000, now Pat. No. 6,399,362, which is a division of application No. 09/092,794, filed on Jun. 5, 1998, now Pat. No. 6,093,557.

(60) Provisional application No. 60/049,444, filed on Jun. 12, 1997.

(51) **Int. Cl.**
B05D 1/06 (2006.01)

(52) **U.S. Cl.** **427/475**; 427/479; 427/483; 427/485

(58) **Field of Classification Search** 118/620–640; 435/285.1, 285.2, 285.3, 458, 459, 470, 471, 435/173.1–173.6; 604/68–72; 239/690, 704–708; 427/475, 479, 483, 485

See application file for complete search history.

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(57) **ABSTRACT**

An electrospraying apparatus and/or method is used to coat particles. For example, a flow including at least one liquid suspension may be provided through at least one opening at a spray dispenser end. The flow includes at least particles and a coating material. A spray of microdroplets suspending at least the particles is established forward of the spray dispenser end by creating a nonuniform electrical field between the spray dispenser end and an electrode electrically isolated therefrom. The particles are coated with at least a portion of the coating material as the microdroplet evaporates. For example, the suspension may include biological material particles.

16 Claims, 10 Drawing Sheets

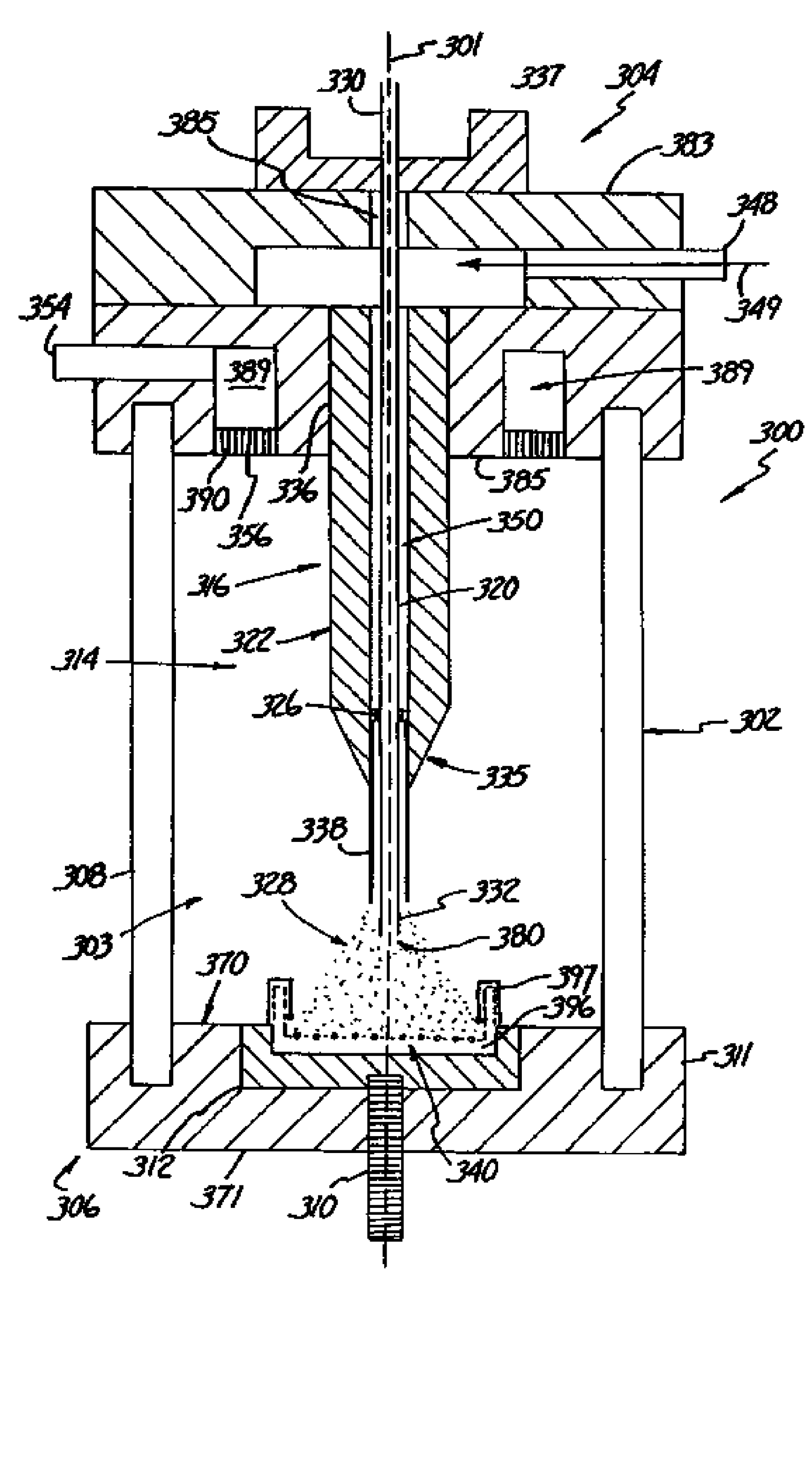

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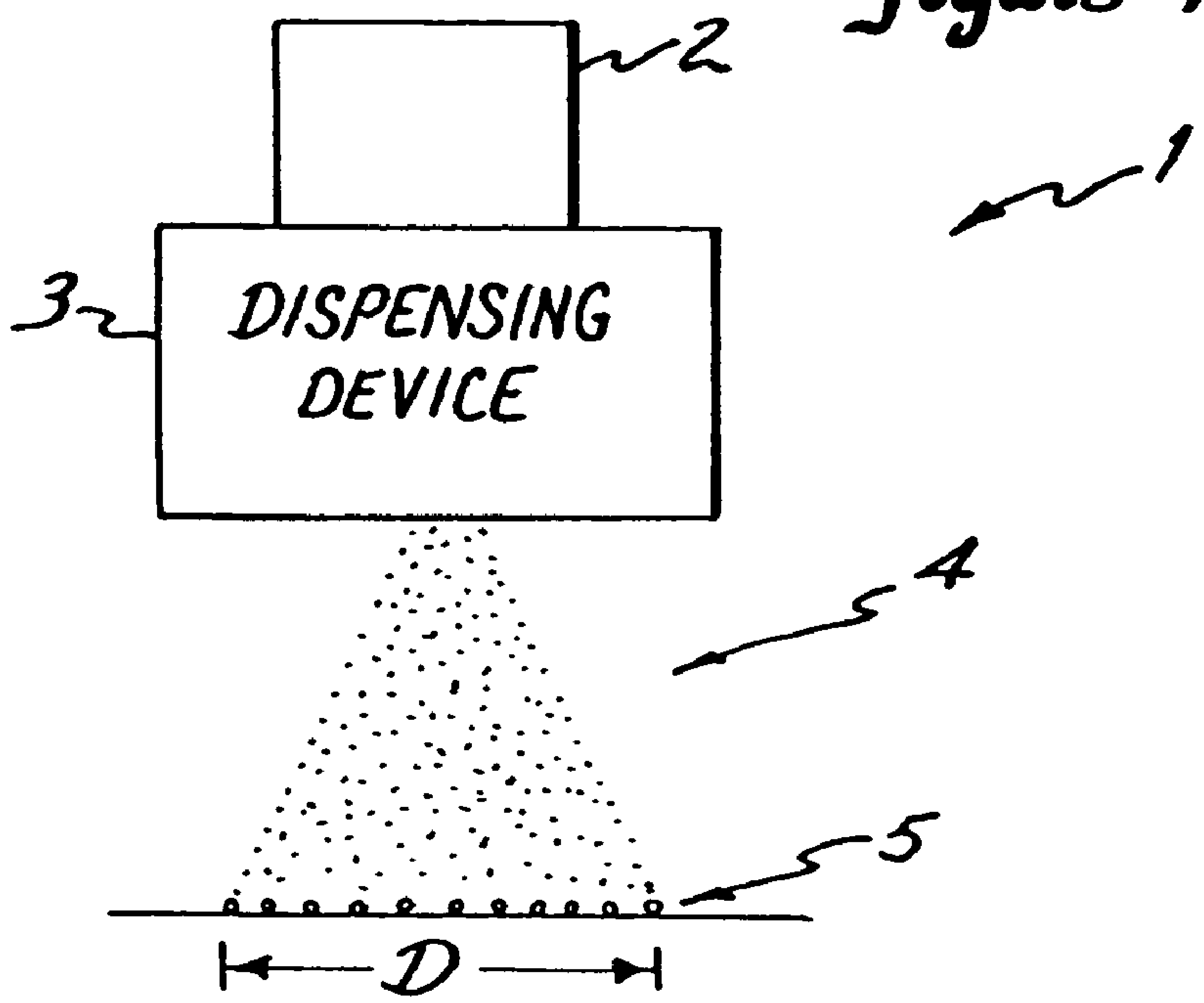
Figure 1A



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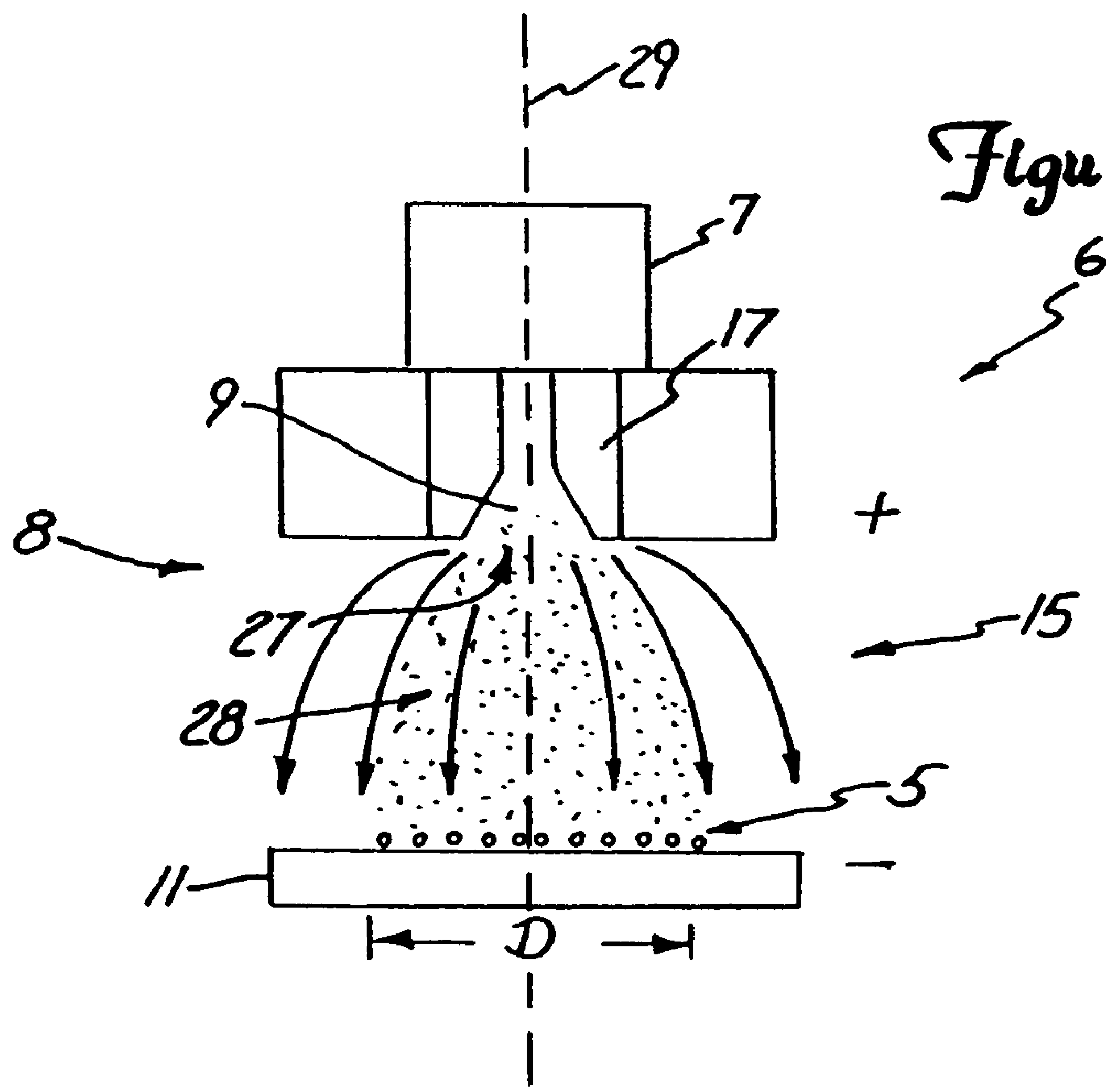

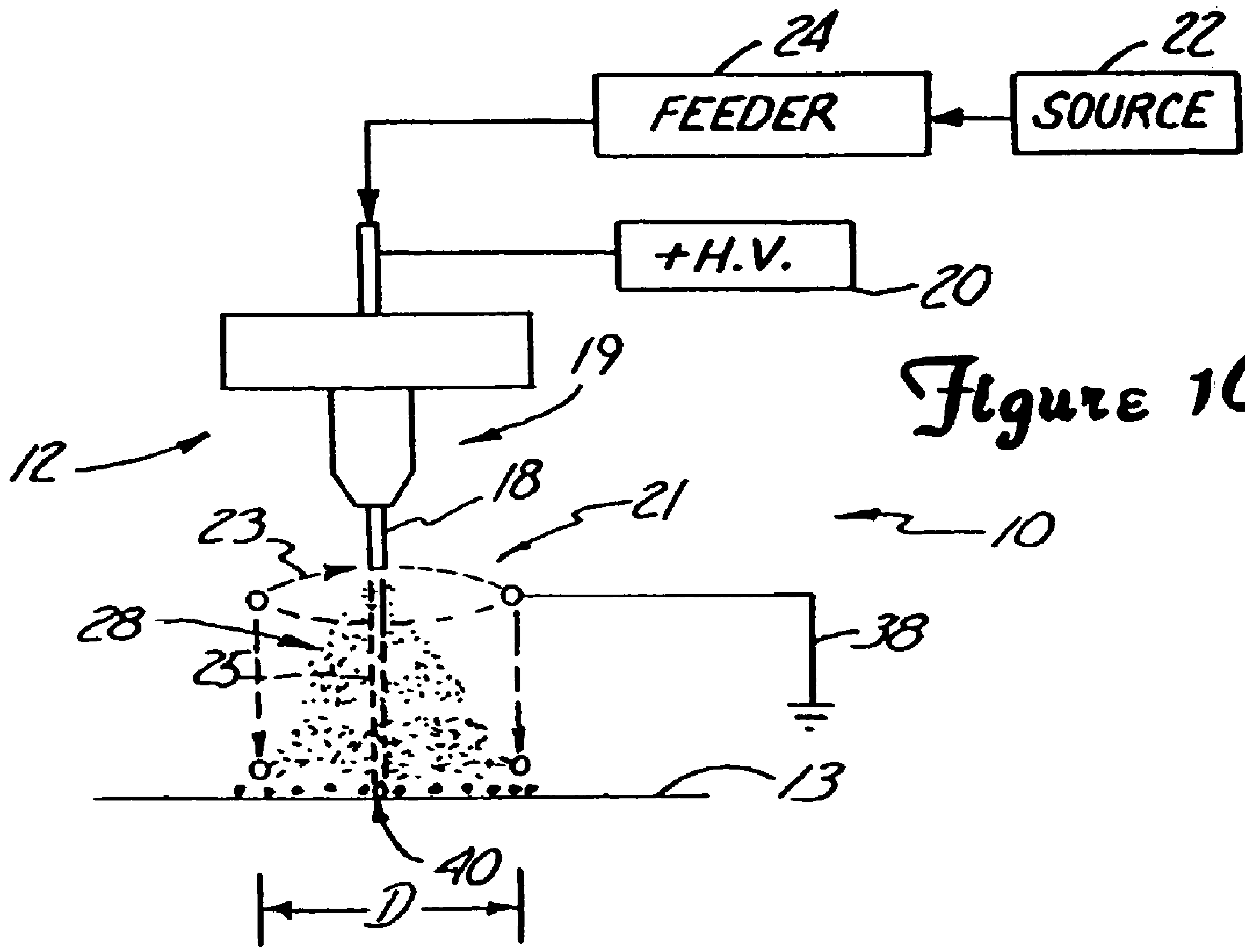

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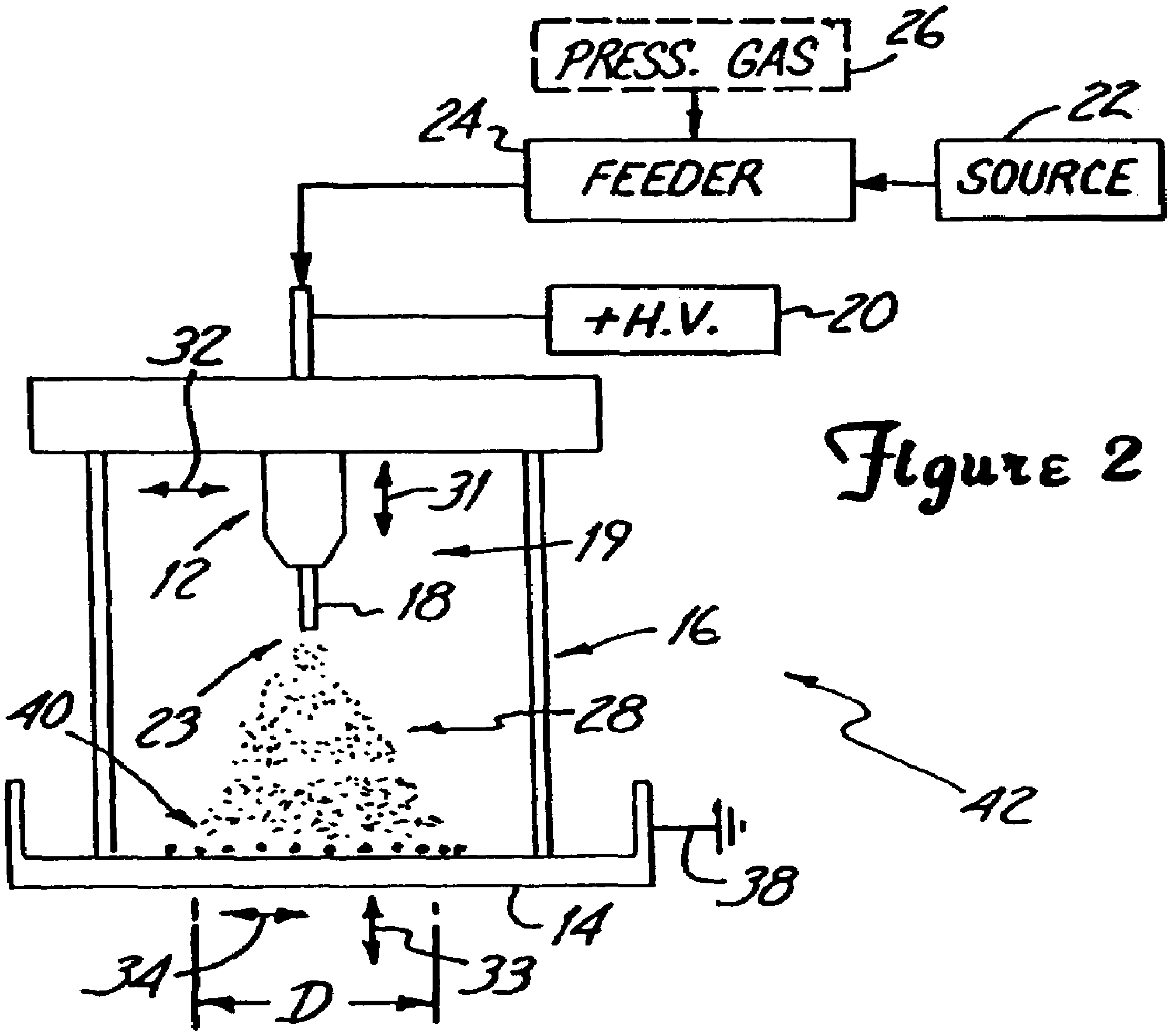
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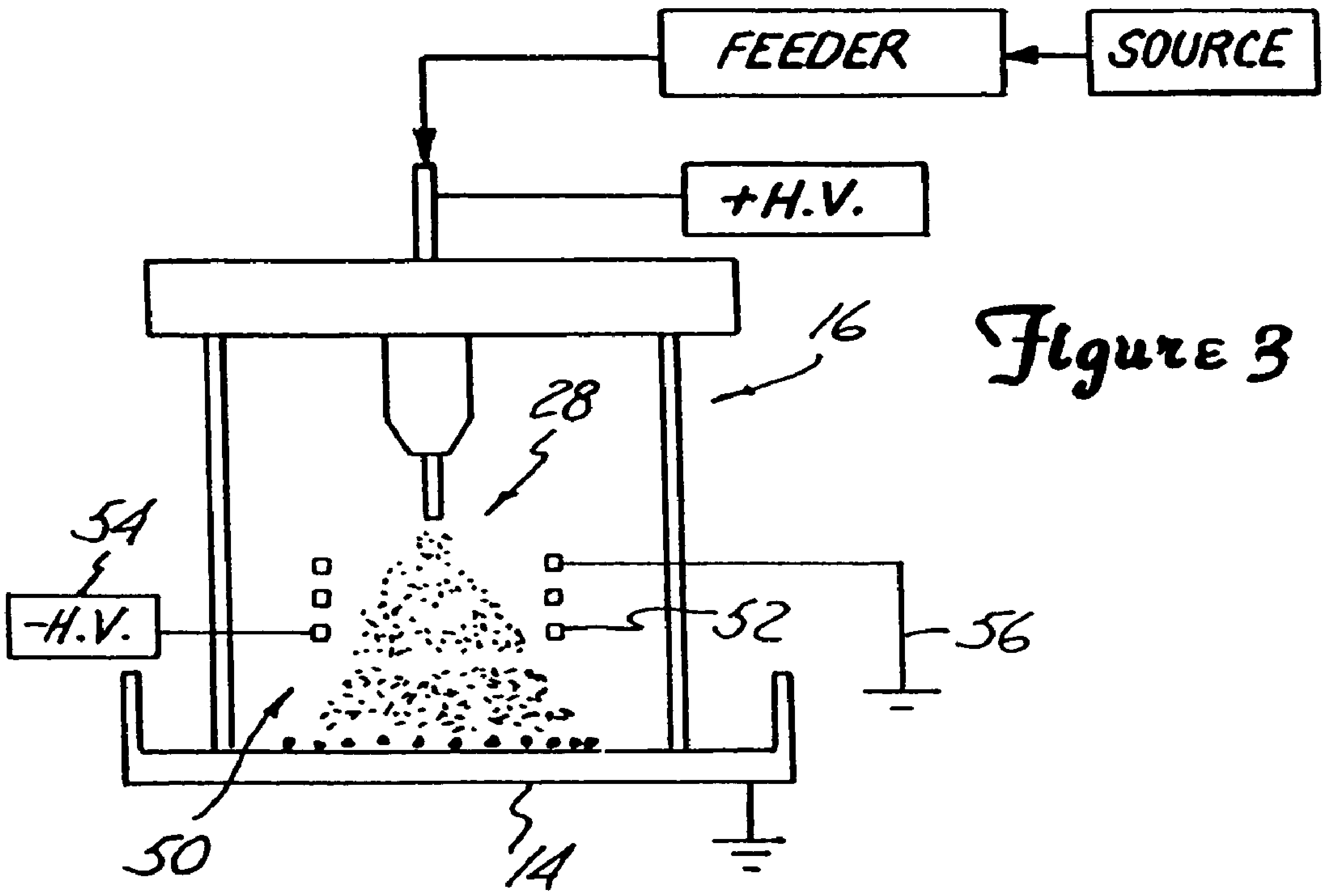

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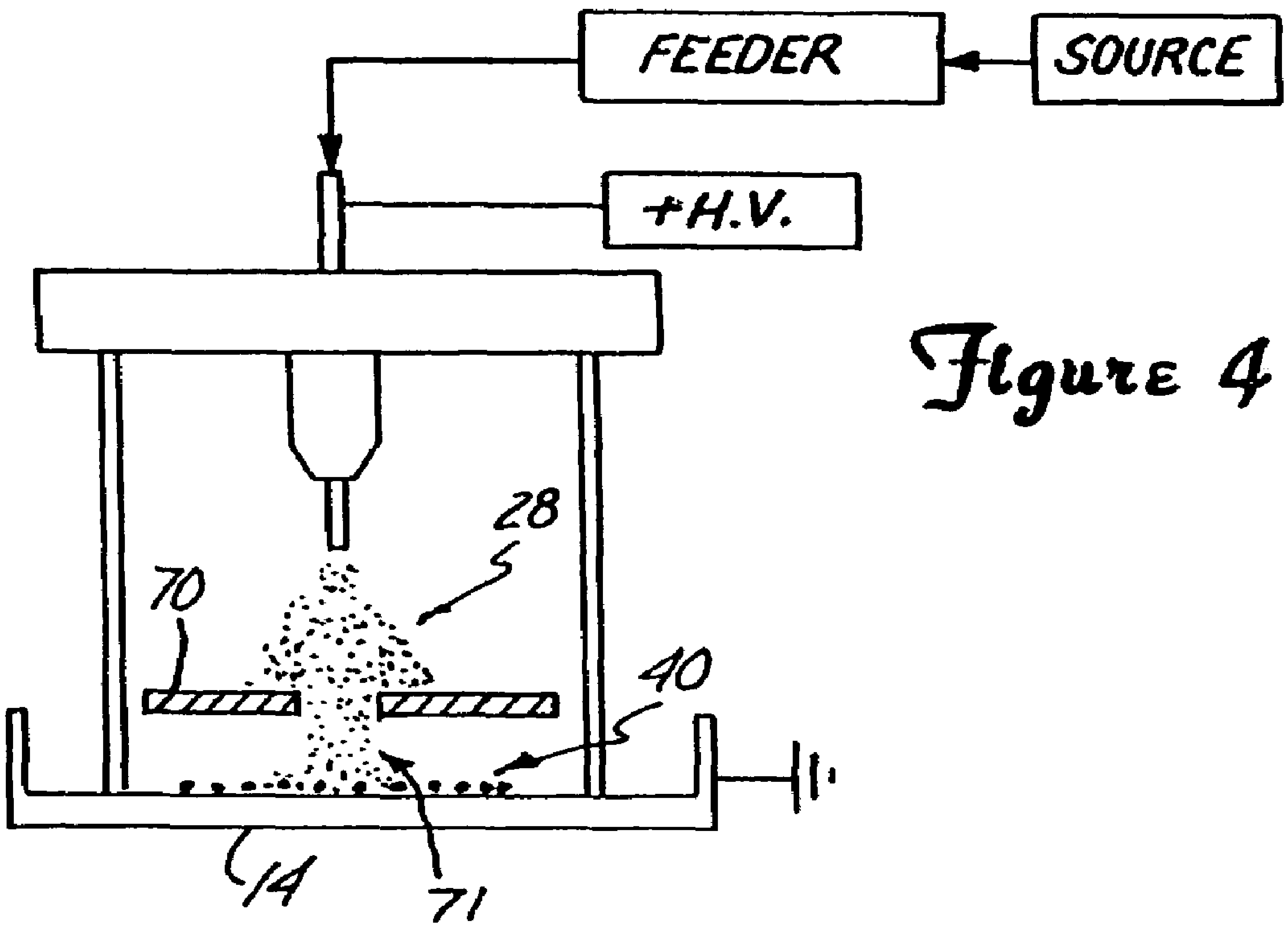

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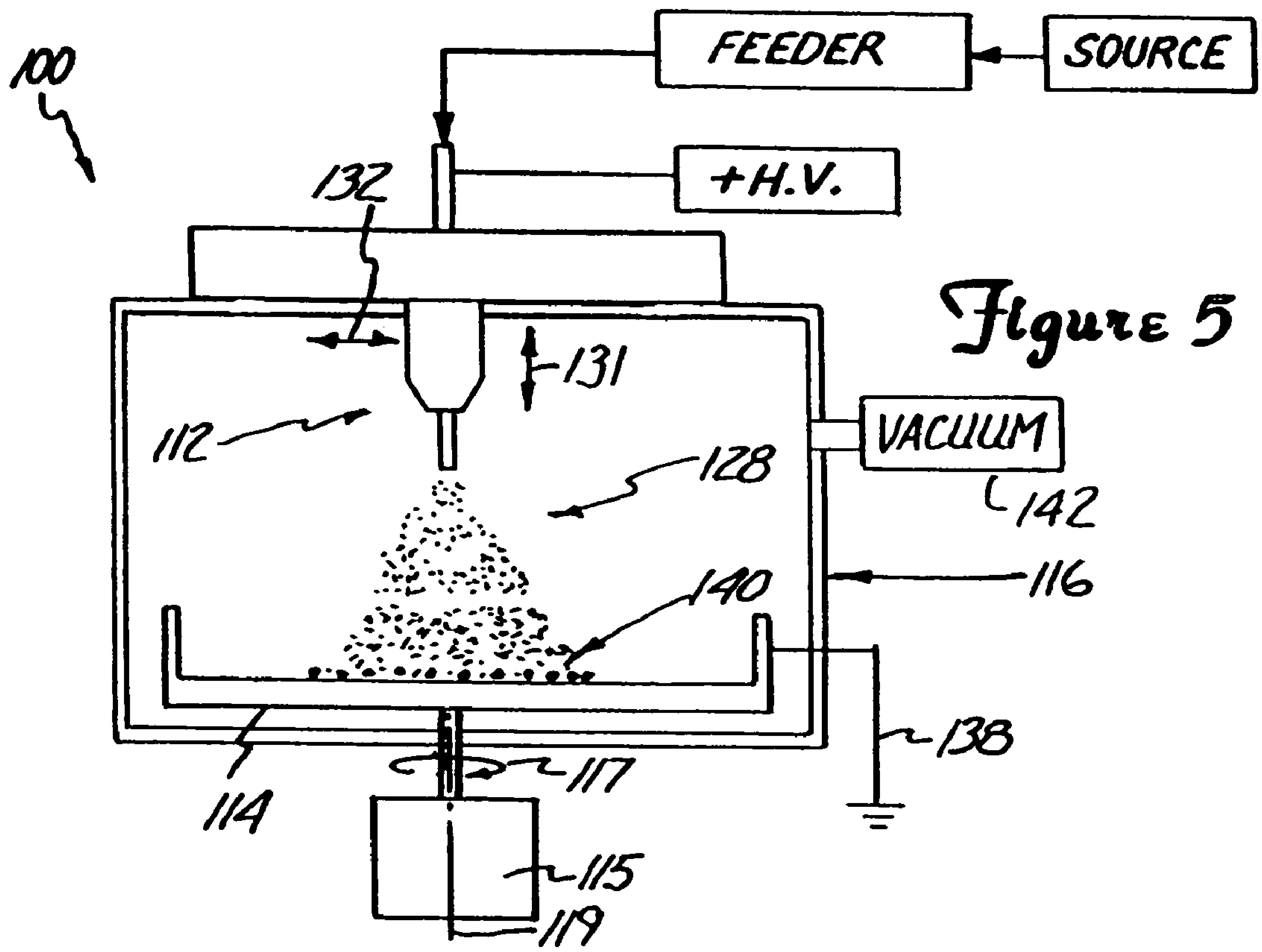
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Figure 5

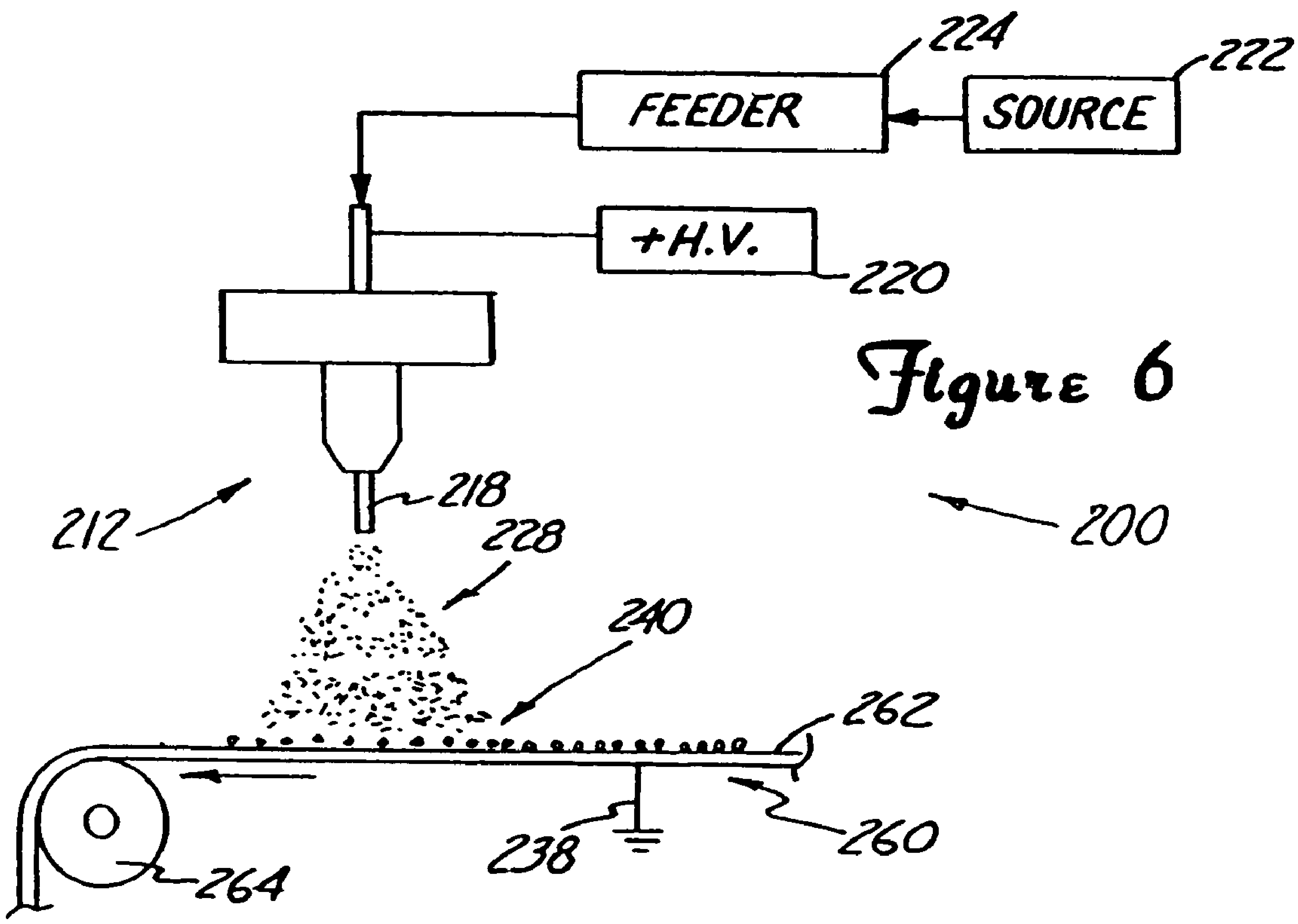
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Figure 6

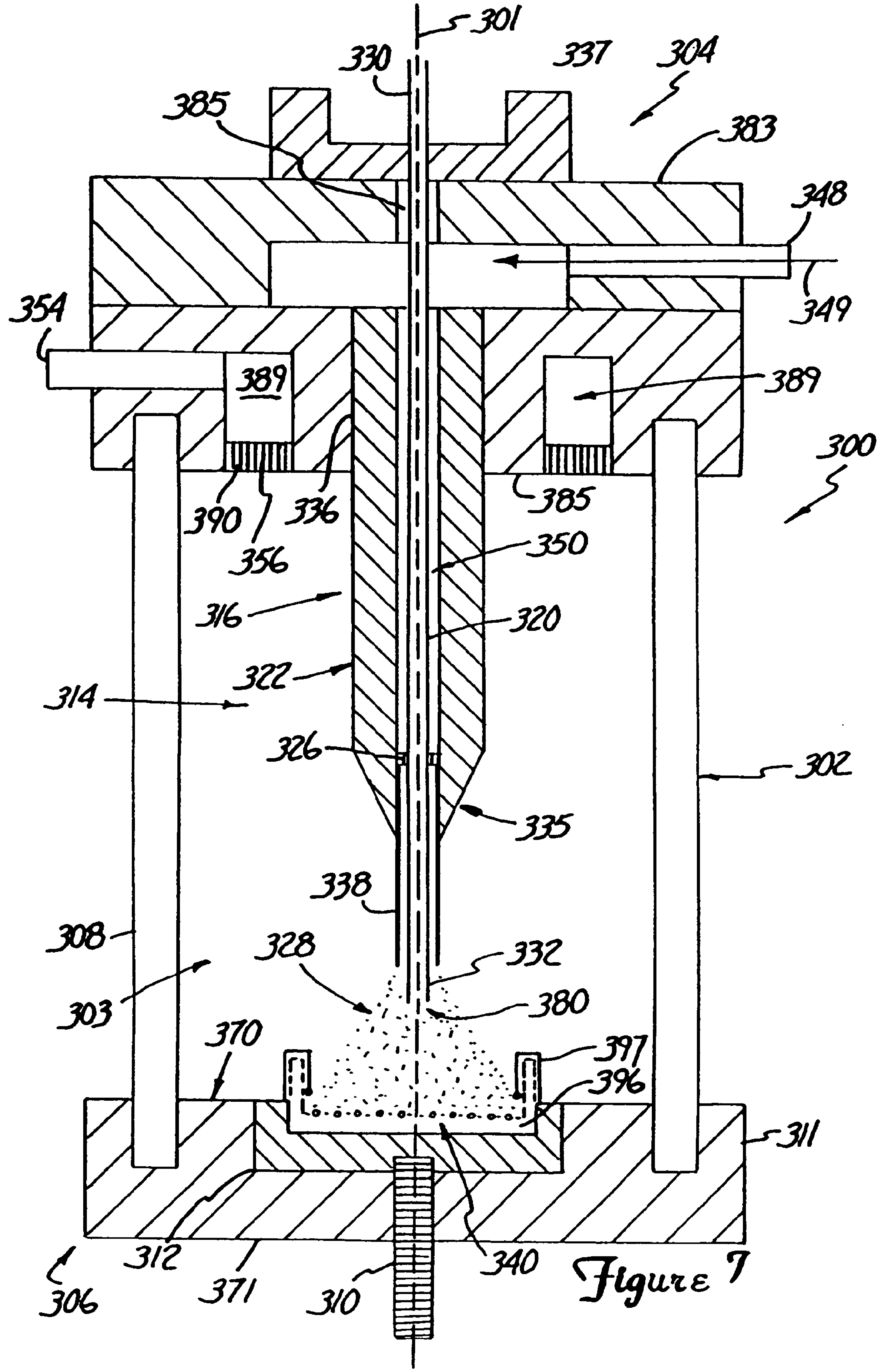
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Figure 7

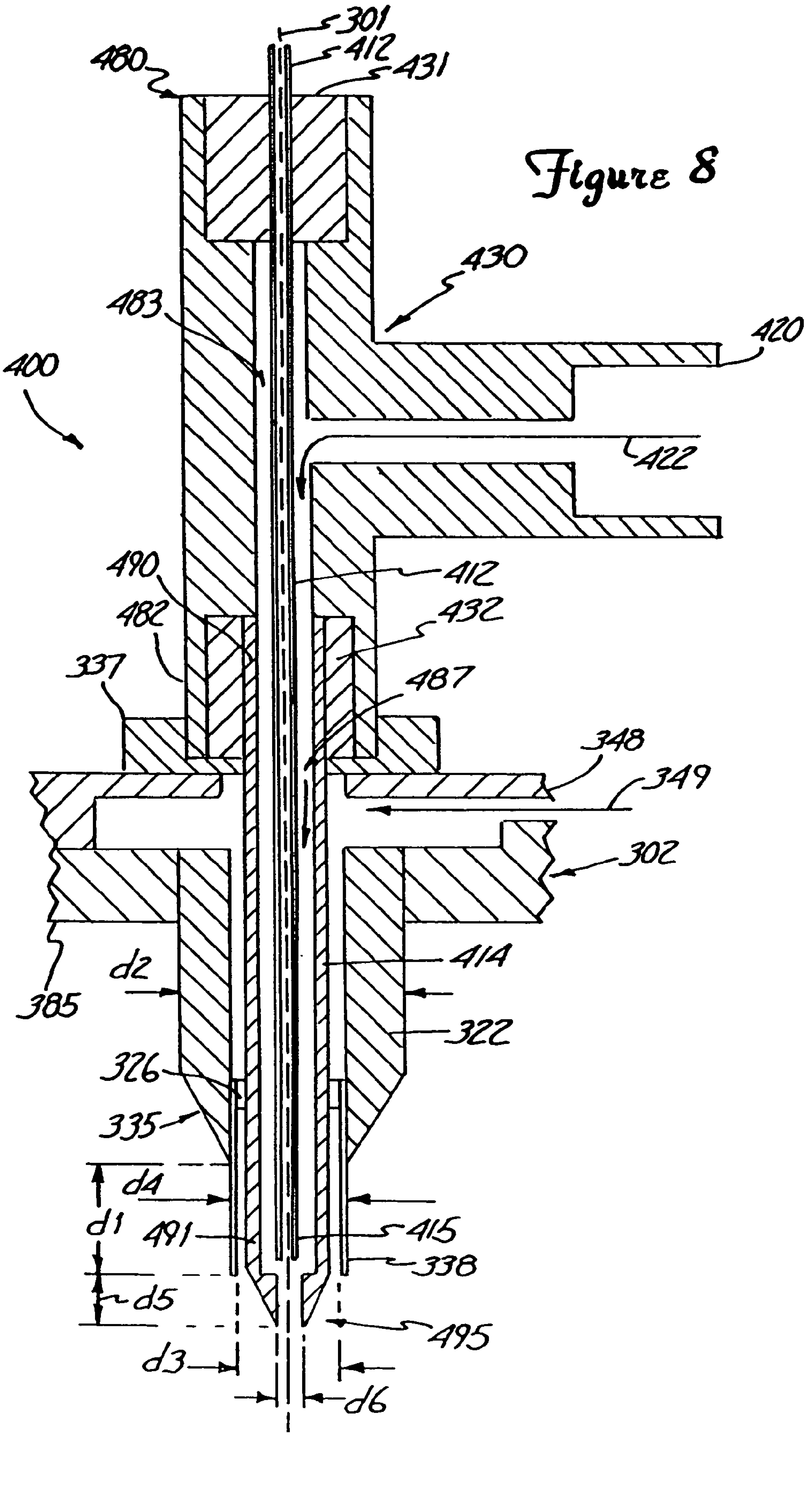

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Figure 8

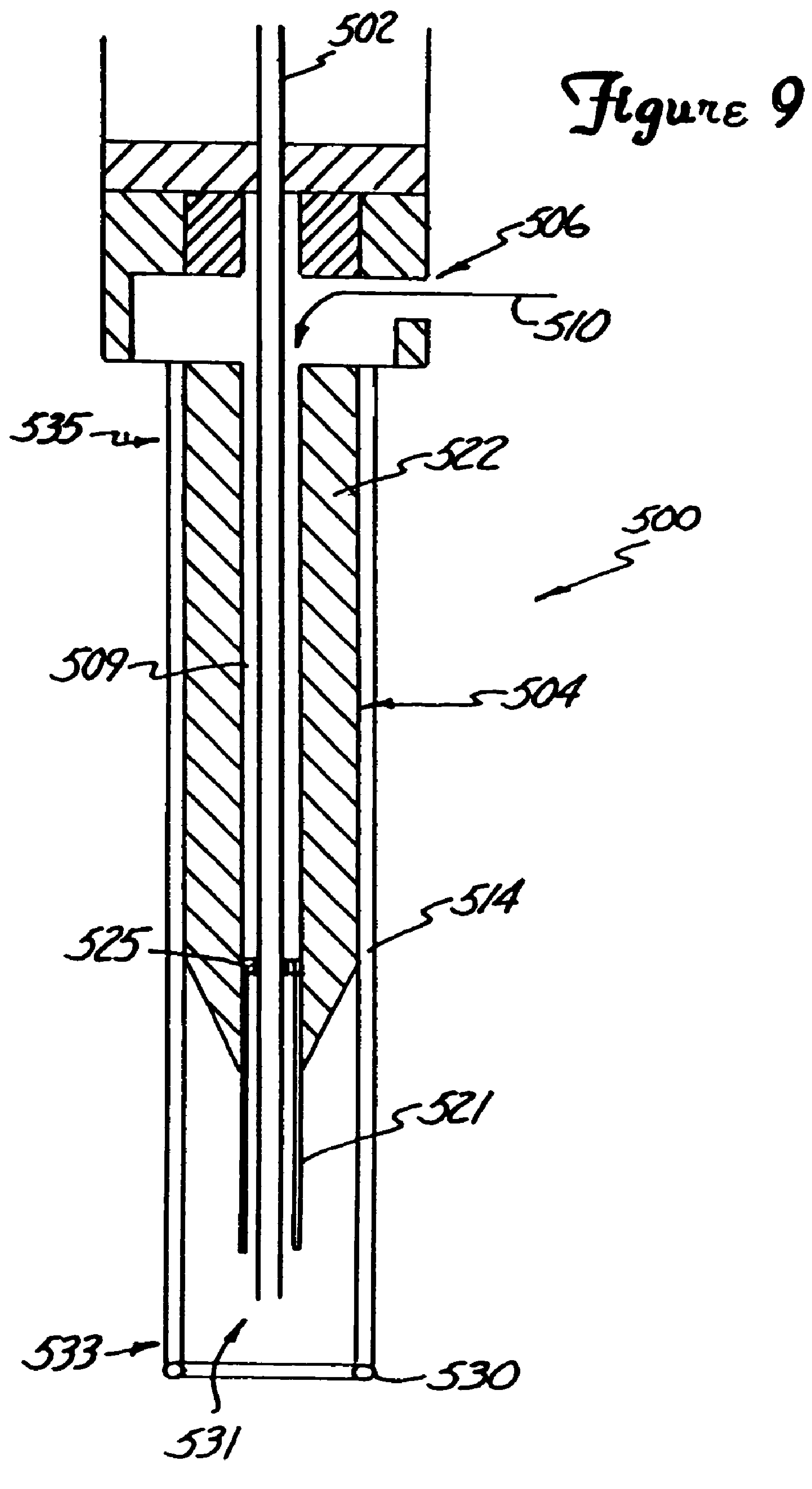
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Figure 9
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ELECTROSPRAYING METHOD WITH CONDUCTIVITY CONTROL

The present application is a continuation application of Ser. No. 10/809,660, filed Mar. 25, 2004, and issued as U.S. Pat. No. 7,279,322 on Oct. 9, 2007, which is a divisional application of Ser. No. 10/162,031, filed Jun. 3, 2002, and issued as U.S. Pat. No. 6,746,869 B2, which is a continuation application of Ser. No. 09/577,747, filed on May 23, 2000, and issued as U.S. Pat. No. 6,399,362, which is a divisional application of Ser. No. 09/092,794, filed on Jun. 5, 1998, and issued as U.S. Pat. No. 6,093,557, which claims benefit of 60/049,444 filed on Jun. 12, 1997, all of which are incorporated by reference herein.

BACKGROUND OF THE INVENTION

Various devices and methods for use in genetic transformation of plant and animal cells have been utilized and many others have been described in various publications. For example, a few early techniques for accomplishing the transport of substances, e.g., DNA, into cells, include uptake mechanisms, fusion mechanisms, and microinjection mechanisms. Generally, uptake mechanisms include the use of substances, such as, for example, liposomes, which encapsulate substances and facilitate transfer of the substances to the cell through fusion of the liposomes with the cell membrane, electroporation, calcium chloride precipitation, and the like. These uptake protocols generally are quite simple and allow for treatment of large numbers of cells at one time, but this technique tends to have a very low efficiency, i.e., transformation frequency is low.

Generally, fusion mechanisms incorporate genetic material into a cell by allowing a cell to fuse with a membrane compatible with the cell membrane of the cell. The fusion of two cells can be used for introducing material into a cell. Cell fusion technologies may have better efficiencies than uptake mechanisms, but cell selection can be more complex, and the resulting cells are typically of elevated ploidy, which makes them of limited use.

Microinjection mechanisms typically employ extremely fine, drawn out capillary tubes, which are sometimes micropipettes or needles. These capillary tubes can be made sufficiently small to be used as syringe needles for the direct injection of biological material into certain types of individual cells. When very small cells are to be injected, very sharp capillary tubes are required, whose tips are very easily broken or clogged. High pressures are required to cause bulk flow through the small orifices and regulation of such flow is difficult. A form of microinjection, commonly referred to as ionophoresis, is also used. Ionophoresis utilizes electrophoresis of substances out of a microelectrode and into a cell, as an alternative to high pressure bulk flow. Although efficiency of microinjection, as one might expect is high, transformation of individual cells is by single cell manipulation and therefore treatment of masses of cells is difficult.

More recently, various techniques involving acceleration of substances for bombardment with cells to accomplish gene transfer have been used and described, e.g., gene guns. For example, such techniques include the use of mechanical impact to project such substances, the use of electrostatic acceleration of the substances, and/or the use of electrostatic discharge to project such substances. It has been stated that such techniques allow the substances to attain a velocity enabling them to penetrate cells.

Various forms of accelerating the substances, for example, are described in the gene gun patent to Sanford et al., U.S. Pat. No. 4,945,050 entitled "Method for Transforming Substances into Living Cells and Tissues and Apparatus Therefor." As described therein, for example, a mechanical shock is applied to a layer of particles (e.g., gold), which are coated, impregnated, or otherwise associated with biological material. The impact causes the particles to be accelerated such that the particles hit the cells to be transformed downstream of the apparatus causing the mechanical shock. The particles puncture the cell membrane and enter the cell, releasing the biological material into the cells.

Spark discharge techniques for accelerating the particles, as described in U.S. Pat. No. 5,120,657 to McCabe et. al., includes the use of a spark discharge chamber. The chamber includes electrodes spaced by a spark gap. A movable particle carrier is moved when a spark discharge in the chamber creates a shock wave that accelerates the movable particle carrier such that the movable particle carrier hits another object accelerating the cells for impact with the target cells to be transformed.

However, such mechanical shock techniques have various disadvantages. First, the techniques are generally batch techniques, i.e., they transfer a certain batch of coated or impregnated particles. If more particles than the number of particles in a single batch are to be transferred, another run or batch must be initiated. For example, this may involve reloading or replacing a part of the apparatus containing the particles, e.g., the movable particle carrier described above.

Further, the coated or impregnated particles when positioned on the transfer surface, e.g., such as the movable particle carrier, may be agglomerated, or such agglomeration may occur during the transfer. Agglomeration of the particles may cause undesirable pit damages to the target cells upon impact therewith.

Yet further, preparation of coated or impregnated particles is a time consuming process. For example, it may take one or more days to precipitate coated or impregnated particles out of a solution containing the carrier particles and the biological material to be transferred.

In addition, the overall process is not easily controlled. For example, there is typically only a limited range of impact velocity which the coated or impregnated particles may attain. The type and origin of the cell can influence the velocity necessary for transformation. Thus, devices that can produce a broader range of impact velocities are desirable. Further, for example, the delivery of particles uniformly to the target cells is not easily controlled. As such, target cells located at certain positions may be damaged more easily than those target cells surrounding such positions. For example, target cells located at the center of a batch of target cells may be damaged or killed more readily than those in the surrounding target area when bombarded by coated or impregnated particles by conventional batch gene gun devices. This may be at least in part due to the agglomeration of the particles. As the overall process is not easily controlled, the amount of biological material being delivered to the target cells is not readily controllable.

Other acceleration techniques, such as aerosol beam technology, electrostatic acceleration fields, centrifugal techniques, etc. as described in U.S. Pat. No. 4,945,050; International Publication WO 91/00915 entitled "Aerosol Beam Microinjector;" and various and numerous other references, may not include all of the disadvantages as described above with regard to the use of mechanical shock. However, such techniques do not alleviate all of such problems. For example, the aerosol technique may allow for a more continuous transfer method as opposed to a batch method, but still has the associated agglomeration disadvantages.

For the above reasons, there is a need in the art for gene transfer methods and apparatus which reduce the effect of such disadvantages as described above. The present invention overcomes the problems described above, and other problems as will become apparent to one skilled in the art from the detailed description below.

SUMMARY OF THE INVENTION

A method of introducing biological material into cells according to the present invention includes providing one or more target cells and establishing a spray of substantially dispersed particles including biological material. The substantially dispersed particles have an electrical charge applied thereto such that one or more of the substantially dispersed particles of the spray is introduced into one or more of the target cells.

In one embodiment of the method, the step of establishing the spray of substantially dispersed particles includes dispensing a spray of microdroplets suspending particles. The electrical charge is concentrated on the suspended particles as the microdroplets evaporate.

In various embodiments, the suspended particles may include carrier particles having biological material associated with the carrier particles or the suspended particles may be particles of biological material. The spray may also be a charged spray of powdered biological material.

Further, in yet another embodiment, the step of dispensing the spray of microdroplets suspending particles may include creating a nonuniform electrical field between a dispensing tip from which the spray is established and an electrode electrically isolated from the dispensing tip. The substantially dispersed particles may be directed towards the one or more target cells using the electrode isolated from the dispensing tip.

In another embodiment, the space charge effect of the concentrated electrical charge on the substantially dispersed particles of the spray enable one or more of the particles to be introduced into one or more of the target cells. The electrical charge concentrated on a particular particle is in the range of about 80 percent to about 95 percent of a maximum charge that can be held by the microdroplet suspending the particular particle.

Yet further, in another embodiment of the method, the step of establishing a spray of substantially dispersed particles includes establishing a continuous spray of substantially dispersed particles.

An apparatus for introducing biological material into one or more target cells according to the present invention includes a biological material source including at least biological material. The apparatus further includes a dispensing device operably connected to the biological material source to receive at least biological material from the biological material source. The dispensing device provides a spray of substantially dispersed particles of at least the biological material. Further, the spray of substantially dispersed particles has an electrical charge applied thereto such that one or more of the substantially dispersed particles of the spray is introduced into one or more of the target cells.

In one embodiment of the apparatus, the biological material source includes a suspension source. The suspension source includes a suspension of at least biological material. Further, the dispensing device receives the suspension and dispenses a spray of microdroplets suspending particles of at least biological material.

In another embodiment, the dispensing device includes a dispensing tip from which the spray of microdroplets suspending particles is dispensed and an electrode isolated from the dispensing tip. A nonuniform electrical field is created between the dispensing tip and the electrode. Generally, the electrode, e.g., a ring electrode or a conductive target surface, is located at a position relative to the dispensing tip to direct the spray of substantially dispersed particles towards the one or more target cells. The target and the dispensing device may be movable relative to each other, e.g., a distance between the dispensing device and the target may be adjusted.

Another apparatus for introducing biological material into target cells according to the present invention includes a biological material source including a suspension of at least biological material. The apparatus further includes a capillary tube electrode. The capillary tube electrode includes a capillary tube having a first open end and a second open end with the capillary tube operatively connected to the biological material source to receive a flow of the suspension of at least biological material at the first open end thereof. The apparatus further includes an electrode isolated from but positioned in proximity to the second open end of the capillary tube. A nonuniform electrical field is created between the capillary tube electrode and the electrode such that a spray of microdroplets suspending particles of at least biological material is provided from the second end of the capillary tube. Further, upon evaporation of the microdroplets an electrical charge is concentrated on the suspended particles resulting in a charged spray of substantially dispersed particles such that one or more of the substantially dispersed particles of the spray is introduced into one or more of the target cells.

In one embodiment of the apparatus, the capillary tube electrode further includes a casing concentric with at least a portion of the capillary tube between the first and second open ends thereof. The second open end of the capillary tube extends beyond the casing a predetermined distance. The apparatus further includes a gas source providing a gas to be received between the capillary tube and the concentric casing.

Yet another apparatus for introducing biological material into target cells according to the present invention is described. The apparatus includes a biological material source including a suspension of at least biological material and an electrolyte source for providing a solution. The apparatus further includes a capillary tube electrode having a dispensing tip. The capillary tube electrode includes a first capillary tube having a first open end and a second open end with the first capillary tube operatively connected to the biological material source to receive a flow of the suspension of at least biological material at the first open end thereof. The capillary tube electrode further includes a second capillary tube concentric with at least a portion of the first capillary tube. The solution is received in an annular opening defined between the first and second concentric capillary tubes. Yet further, the apparatus includes an electrode isolated from but positioned in proximity to the dispensing tip of the capillary tube electrode. A nonuniform electrical field is created between the capillary tube electrode and the electrode such that a spray of microdroplets suspending particles of at least biological material is provided from the dispensing tip. Upon evaporation of the microdroplets, an electrical charge is concentrated on the suspended particles resulting in a charged spray of substantially dispersed particles.

Another method for introducing biological material into target cells according to the present invention includes providing one or more target cells, providing a first flow of a suspension including at least biological material, and providing a second flow of electrolyte solution. A spray of substantially dispersed particles including at least biological material is established from the first flow and the second flow. The substantially dispersed particles have an electrical charge applied thereto such that one or more of the substantially dispersed particles of the spray is introduced into one or more of the target cells.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A is a general block diagram representative of an electrospraying apparatus in accordance with the present invention for establishing a charged spray using a biological material source.

FIG. **1**B is a general diagrammatical illustration of an electrospraying apparatus in accordance with the present invention for establishing a charged spray using a biological material source including a suspension.

FIG. **1**C is one embodiment of the electrospraying apparatus of FIG. **1**B in accordance with the present invention for establishing a charged spray using a capillary tube electrode and a biological material source including a suspension.

FIG. **2** is a diagrammatical illustration of another embodiment of an electrospraying apparatus of FIG. **1**B in accordance with the present invention.

FIG. **3** is a diagrammatical illustration of the apparatus shown in FIG. **2** including an additional electrostatic acceleration field.

FIG. **4** is a diagrammatical illustration of the apparatus of FIG. **2** including a placement control member.

FIG. **5** is a diagrammatical illustration of an alternate electrospraying apparatus in accordance with the present invention using a vacuum chamber.

FIG. **6** is a further alternate electrospraying apparatus in accordance with the present invention illustrating a continuous electrospraying apparatus.

FIG. **7** is a more detailed diagram of a portion of the electrospraying apparatus in accordance with the present invention having a single capillary tube distributor head.

FIG. **8** is a more detailed diagram of an alternate capillary configuration for use in the apparatus shown in FIG. **7** including a dual capillary tube distributor head.

FIG. **9** shows an illustrative diagram of a portion of a compact pen-like electrospraying apparatus in accordance with the present invention.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present invention shall first generally be described with reference to FIGS. **1**A-**1**C. Various other embodiments of the present invention shall then be described further with reference to FIGS. **2**-**9**. It will become apparent to one skilled in the art that elements from one embodiment may be used in combination with elements of the other embodiments and that the present invention is not limited to the specific embodiments described herein but only as described in the accompanying claims.

The present invention is directed to apparatus and methods for introduction of biological materials, such as, for example, DNA, into target cells, e.g., plant or animal cells. As shown in FIG. **1**A, the present invention uses an electrospraying apparatus **1** to establish a spray **4** of charged particles. The electrospraying apparatus **1** includes a dispensing device **3** which receives at least biological material from a biological material source **2** and establishes the charged spray **4** forward thereof. The space charge effect of the charged particles of the spray **4** enable the particles to attain a velocity such that the particles forcibly contact, and preferably, penetrate target cells **5** when impacted.

As used herein the term charged spray **4** shall refer to a spray of particles having a charge applied thereto established from a source of biological material **2**. The source of biological material may be a source of dry biological material alone or biological material associated with carrier particles, i.e., a powdered form of biological material. Preferably, the source of biological material **2** is a suspension of biological material, i.e., a solution including at least biological material. For example, the suspension of biological material may be a suspension of biological material alone or a suspension of biological material and carrier particles. However, any source of biological material which can be sprayed with a unipolar charge (i.e., a same polarity charge) applied thereto can be used according to the present invention.

The dispensing device **3** may be any device for establishing a spray of charge particles **4** with a unipolar charge applied thereto such that the space charge effect of the charged particles of the spray **4** enable the particles to attain a velocity allowing the particles to forcibly contact, and preferably, penetrate the target cells **5**. The configuration of the dispensing device **3** will depend at least upon the type of biological material source **2** used. For example, when the biological material source **2** is a source of dry biological material alone or biological material associated with carrier particles, i.e., a powdered form of biological material, the dispensing device **3** may take the form of a spraying device which applies a unipolar charge to the particles of the spray using corona discharge. Such a spraying device may include a structure having an orifice therethrough. A flow of the powdered material may be provided through the orifice, e.g., by way of a pressurized gas source. Upon exit from the orifice, the particles of the spray may be subjected to a weak corona established by brushes positioned about the orifice. One skilled in the art will recognize that this is just one illustrative example of a device for spraying powdered biological material with a charge applied thereto and that the present invention is clearly not limited to this particular embodiment but is limited only as described in the accompanying claims.

As one skilled in the art will recognize, as used herein, the term applied charge refers to applying a unipolar charge (e.g., the same polarity charge) to the particles of spray **4**. For example, the charge may be applied by corona discharge as described above with reference to powdered biological material. Further, for example, the charge may be applied by concentration of charge on the spray of particles through evaporation of solution suspending the particles in an established electrical field as further described below with respect to the general illustration of FIG. **1**B. In other words, for example, the biological material source **2** is a suspension of biological material and a spray of microdroplets is dispensed from the dispensing device **3**, i.e., particles suspended by microdroplets are dispensed. The particles suspended by microdroplets may be carrier particles and biological material or biological material itself without the use of carrier particles. In other words, when dispensed, the spray is preferably a spray of liquid suspended particles as opposed to a powder spray. The liquid portion of the spray of suspended particles generally evaporates to concentrate the charge of the liquid portion on the particles resulting in a spray of charged particles as will be described further below with reference to FIG. **1**B.

The spray of particles provided by electrospraying provides for a controllable biological material transfer process which is not limited to batch processing. Rather, the electrospray technique may be utilized in a continuous manner.

The electrospraying mechanism **1** provides a charged spray with a high concentration of charged particles. Preferably, the concentration of charged particles in the spray is in the range of about 10^5 particles/cubic centimeter (articles/cc) to about 10^{12} particles/cc; more preferably in range of about 10^7 particles/cc to about 10^{10} particles/cc; and further even more preferably about 10^9 particles/cc. Below about 10^5 particles/cc, the concentration of charged particles is to low for the space charge effect to attain a velocity for introduction into most target cells. Due to the space charge effect, i.e., the effect created by the charge repulsion of charged particles, a spray of substantially dispersed particles having the same polarity charge is provided with the particles distributed substantially uniformly across the spray area (e.g., the area represented by D in FIG. **1**A) wherein the target cells are placed. As used herein, the term substantially dispersed particles refers to uniformly and/or nonuniformly sized particles separated by an applied repulsive electrostatic force. Thus, the electrospray process is a consistent and reproducible transfer process. Further, because the charged particles of the spray repel from one another, agglomeration of the particles is avoided. As such, the electrospray technology provides for reduced cell pit damage and shock injury which are common results when particle agglomeration occurs utilizing conventional methods of transfer. In addition, as described below, the electrospraying technique allows the gene transfer process to be controlled in various manners.

Due to the small size of the charged particles of the spray established in the region of a target including one or more cells, the space charge effect, i.e., the effect created by the charge repulsion of charged particles, provides particles having sufficient velocity to forcibly contact, and preferably penetrate, one or more target cells. However, such space charge effect also creates a spray of charged particles that is generally not contained, i.e., the particles randomly disperse in multiple directions. Therefore, it is preferable to confine or direct the spray of charged particles towards the one or more target cells. As illustrated below, one technique of providing such containment and/or direction for such charged particles is to use an electrode already required to establish the charged spray when the biological material source is a suspension of at least biological material. In other words, the electrode is used to provide a nonuniform electric field for establishing a charged pray and also provides direction for the particles of the charged spray as is described further below.

FIG. **1**B generally shows a diagrammatical illustration of an electrospraying apparatus **6** for establishing a charged spray **28** using a dispensing device **8** which receives a flow of a suspension from a biological material source **7**. The biological material source **7** contains a suspension of at least biological material, e.g., biological material alone or biological material and carrier particles. Generally, the dispensing device **8** includes a conductive structure **17** defining an orifice **9** (e.g., a capillary tube, an orifice defined in a flooding chamber, etc.) for receiving a flow of solution suspending particles, e.g., biological material alone or carrier particles along with biological material. For example, the solution may be pushed or pulled through the orifice **9** at dispensing tip **27** of the conductive structure **17** defining the orifice **9**, e.g., pushed by a pump. The conductive structure **17** defining the orifice **9** functions as a first electrode of the dispensing device **8** with the dispensing tip **27** of the conductive structure positioned for dispensing microdroplets towards target cells **5**. Further the dispensing device **8** includes a second electrode structure **11**. An electrical potential difference is applied between the first electrode **17** and second electrode **11** to create a nonuniform electric field between the first electrode **17** and second electrode **11**. One skilled in the art will recognize that the electrodes may be formed using one or more conductive elements.

Generally, in operation, a flow of the suspension is provided through the orifice **9**, e.g., pushed and/or pulled through the orifice **9**. A meniscus is formed at the dispensing tip **27** where the orifice **9** has a diameter in the preferred range of about 6 microns to about 2 millimeters (mm). A potential difference is applied to establish a nonuniform field **15** between the first electrode **17** and second electrode **11**. For example, a high positive voltage may be applied to the first electrode **17** with the second electrode **11** being grounded. Further, for example, a voltage difference in the preferred range of about 2000 volts to about 6000 volts may be applied.

As used herein, nonuniform electric field refers to an electric field created by an electrical potential difference between two electrodes. The nonuniform electric field includes at least some electric field lines that are more locally concentrated at one electrode relative to the other electrode, e.g., more concentrated at the dispensing tip relative to the second electrode. In other words, for example, at least some of the field lines are off-axis relative to a longitudinal axis **29** through the center of the orifice **9**. Further, for example, the electrode **11** is positioned forward of the dispensing tip **27** towards the target cells **5** and the electrode **11** is of a size and/or includes at least a portion that is located at a position away from the longitudinal axis **29**. Yet further, for example, the electrode **11** may be a ring electrode having a diameter larger than the diameter of the orifice **9** and positioned forward of the dispensing tip **27** with an axis through the center of the ring electrode coincident with the longitudinal axis **29** of the orifice **9**. Further, for example, the electrode **11** may be a conductive target surface having an area greater than a cross section area taken through the orifice **9** perpendicular to the longitudinal axis **29** and positioned forward of the dispensing tip **27**.

In the case where the biological material source **7** is a suspension of biological material (without the use of carrier particles), the suspension is flowed (e.g., pushed and/or pulled) through the orifice **9**. Generally, the liquid portion of the suspension provided to the orifice **9** has an electrical conductivity. The biological material generally has a small charge associated therewith, e.g., DNA may have a small negative charge, but the charge of the biological material is inconsequential due to the larger charge concentrated on the biological material as described below.

As the suspension progresses through the orifice, the potential difference between the first and second electrodes which creates the electrical field therebetween strips the liquid of one polarity of charge, i.e., the negative charge is stripped when a high positive voltage is applied to the electrode **17**, leaving a positively charged microdroplet to be dispensed from the dispensing tip **27**. For example, the meniscus at the dispensing tip may form a cone jet for dispensing a spray of microdroplets suspending biological material when the forces of the nonuniform field **15** balances the surface tension of the meniscus. The spray of microdroplets further become more positive in the nonuniform electric field **15**.

As the microdroplets evaporate, the charge of the microdroplets concentrate on the biological material resulting in a spray of charged biological material particles. The amount of charge on the microdroplet, and thus the amount of charge on a particle after evaporation, is based at least upon the conductivity of the liquid used to spray the microdroplets, the surface tension of the liquid, the dielectric constant of the liquid, and the feed flow rate of the liquid. Generally, the space charge effect due to the concentrated electrical charge on the substantially dispersed particles of the spray enable the particles to forcibly contact, and preferably, penetrate the target cells. The electrical charge concentrated on a particular particle is preferably in the range of about 80 percent to about 95 percent of a maximum charge that can be held by the microdroplet suspending the particular particle, e.g., biological material particle, without the microdroplet being shattered or torn apart, i.e., in the range of about 80 percent to about 95 percent of the Rayleigh charge limit. At 100 percent, the surface tension of the microdroplet is overcome by the electrical forces causing droplet disintegration. The nonuniform electrical field also provides for containment of the particles and/or direction for the particles which would otherwise proceed in random directions due to the space charge effect.

In the case where the biological material source **7** is a suspension of biological material and carrier particles, the suspension is flowed (e.g., pushed or pulled) through the orifice **9**. Generally, the liquid portion of the suspension provided to the orifice **9** has an electrical conductivity. As will be described below, more than one flow of solution may be used to establish the spray. For example, one flow of material may be a suspension of material using deionized water with a second flow of material including an electrolyte solution having a suitable conductivity. The biological material generally has a small but inconsequential charge associated therewith. The carrier particles are generally neutral.

As the suspension progresses through the orifice, the potential difference between the first and second electrodes which creates the nonuniform electrical field therebetween strips the liquid of one polarity of charge, i.e., the negative charge is stripped when a high positive voltage is applied to the electrode **17**, leaving a positively charged microdroplet to be dispensed from the dispensing tip **27**. A spray of microdroplets suspending biological material and carrier particles is established forward of the dispensing tip **27** with the microdroplets being positively charged.

As the microdroplets evaporate, the charge of the microdroplets concentrate on the biological material and carrier particles resulting in a spray of positively charged carrier particles associated with biological material. The biological material, which may carry a slightly negative charge, are attracted to the positively charged carrier particles resulting in better adhesion between the biological material and the carrier particles. This is unlike conventionally prepared carrier particles having associated biological material because in conventional processes the neutral carrier particles do not create such attraction forces with the slightly negatively charged biological material. In other words, the present invention provides a better coating process for coating carrier particles with biological material. This results in more uniform distribution of biological material being delivered to the target cells. Generally, as described above, the space charge effect due to the concentrated electrical charge on the substantially dispersed particles of the spray enable the particles to forcible contact, and preferably, penetrate the target cells.

One skilled in the art will recognize that the voltages applied may be reversed. For example, the first electrode may be grounded with a high positive voltage applied to the second electrode. In such a case, the particles of the spray would have a negative charge concentrated thereon. Further, any other applied voltage configuration providing a nonuniform electrical field to establish the charged spray of particles may be used.

Further, one skilled in the art will recognize that the spray of particles need not have the biological material associated with the carrier particles. For example, if a positive voltage is applied to the second electrode **11** and the first electrode **17** is grounded, then the carrier particles which are normally neutral in the suspension will have a negative charge thereon as they are spray. With biological material being slightly negative, repulsion forces may keep the carrier particles separated from the biological material and therefore unassociated therefrom. In such a manner, for example, the carrier particles and the biological material particles would be separate from one another in the spray of charged particles. The carrier particles may penetrate the target cells first forming a channel in the target cells such that the biological material particles may easily travel therethrough for introduction into the target cells.

One generalized embodiment of the electrospray apparatus **6** shown generally in FIG. **1**B shall be described with reference to the electrospraying apparatus **10** shown in FIG. **1**C. Generally, the electrospraying apparatus **10** in accordance with the present invention includes an electrospray dispensing device **12** positioned for providing a charged spray **28**. Downstream from or forward of the dispensing device **12** is positioned a target **13** including one or more target cells **40**.

In accordance with the present invention, the spray **28** has an electrical charge applied thereto by way of a high positive voltage source **20** applied to a capillary tube electrode **18** of distributor head **19** of the electrospray dispensing device **12** and the electrode **21** being connected to ground **38**. The spray **28** is established as described above with use of the nonuniform electric field created between the dispensing tip **23** of the capillary tube electrode **18** and the electrode **21**. The spray **28** may be provided by any electrospray dispensing device suitable for providing a spray **28** having a charge applied thereto. Preferably, the charge of the particles is adequate for enabling the particles of the spray **28** to have a velocity due to space charge effect sufficient for the dispersed particles of the spray **28** to penetrate target cells **40**.

The particle velocity is primarily a function of the particle charge and the space charge effect. The nonuniform electric field formed between the high voltage capillary tube electrode **18** and the electrically grounded electrode **21** provides for the dispensing of the spray **28** from the dispensing tip **23** of distributor head **19**. As described below, depending upon the potential difference applied between the distributor head **19** having a first electrode, e.g., capillary tube electrode **18**, and the second electrode **21**, different modes of spray operation can be established.

The nonuniform electric field can be provided by various configurations. For example, the second electrode **21** may be any conductive material grounded and positioned to establish the formation of a spray **28** from the dispensing tip **23** of the distributor head **19** or otherwise causing the provision of a charged spray from the distributor head **19**, e.g., the second electrode may be a grounded ring electrode, a grounded target surface holding the cells, etc. The second electrode **21** may be located at various positions as shown in FIG. **1**C. For example, the electrode **21** may be located at a position just forward of the distributor head **19**, or the electrode **21** may be located further away from the distributor head **19** closer to the target cells **40**.

It will be recognized that the second electrode **21** may take one of many different configurations. For example, the electrode may be a conductive platform upon which the cells are positioned. Further, for example, the electrode **21** may be a ring electrode having an axis coincident with an axis of distributor head **19**, etc. For the electric field to be nonuniform, at least one portion of the electrode **21** must be positioned outside of a hypothetical cylinder **25** extending from the perimeter of the capillary tube electrode to target **13**. In other words, electric field lines must extend to and/or from an area outside of the hypothetical cylinder **25**.

The strength of the field may be adjusted by adjustment of the distance between the first electrode **18** and second electrode **21**. The farther the electrode **21** is from the distributor head **19**, the lesser the field strength. However, with such increasing distance, more directionality is provided for the spray **28**. For example, if the second electrode **21** is close to the distributor head **19**, the space charge effect will cause the particles to disperse into a relatively large area D. On the other hand, the particles can be directed to various targets by moving the electrode **21** to various positions. For example, the electrode **21**, e.g., a ring electrode, can be moved close to the target cells **40** to provide a uniform spray **28** in the area proximate thereto.

The source **22** which provides biological material to feeder **24** may be one of many types of biological material sources. Source **22** may be a liquid suspension including biological material. Further, the liquid suspension may include a liquid suspension of bulk biological material (i.e., without carrier particles), may be a liquid suspension of carrier particles and biological material, or may be a liquid suspension of carrier particles having biological material associated therewith, e.g., carrier particles coated or impregnated with DNA.

The present invention hereinafter shall primarily be described with reference to use of a source **22** that is a suspension of carrier particles and biological material, e.g., DNA and gold particle suspension. However, even though the description is focused to the use of a carrier particle suspension, the benefits of the present invention are clearly applicable when other sources are used for providing charged sprays as described herein. It will be recognized that carrier particles of the suspension of carrier particles and biological matter need not be coated with the biological material prior to preparing and using the suspension. In other words, generally, such a suspension is created by mixing the carrier particles and biological material into the suspension liquid, e.g., buffer, electrolyte solution, deionized water, etc. This generally eliminates the substantially time consuming conventional preparatory processes involved in coating or impregnating carrier particles for use in conventional batch gene gun devices.

The suspension may include any liquids suitable for biological material delivery. Further, a component of calcium chloride may be used in the liquid. Any solutions which are suitable for raising cells, such as nutriant solutions, may also be used. Further, for example, the liquid used in the suspension may be deionized water when an additional conductive liquid is used therewith or when another flow of electrolyte solution is used with the flow of suspension to establish the spray of particles.

As known to one skilled in the art, various inert particles may be used as the carrier particles. For example, such inert carrier particles may include ferrite crystals, gold, tungsten spheres, and other metal spheres, as well as spheres and particles such as glass, polystyrene, and latex beads. Preferably, the carrier particles are only mixed in the suspension with the biological material. However, such carrier particles may be coated or impregnated with biological material or otherwise associated therewith. For example, biological material may be coated on, bonded on, or precipitated onto the surface of the carrier particles or impregnated with the biological material. As described above, the carrier particles generally become associated with the biological material as the suspension is spray. The carrier particles act as the carrier for carrying the biological materials into the target cells. When one or more carrier particles having biological material associated therewith penetrate the cell membrane of the target cells, the biological material is dispersed within the cell.

Biological material which can be used with the inert carrier particles include but are not limited to biological stains such as fluorescent or radio-labeled probes, viruses, organelles, vesicles, peptides, ammoacids, lipids, proteins such as enzymes or hormones, nucleic acids, polynucleic acids including DNA and RNA, individual nucleic acids, small molecules such as bioactive substances, drugs, or the like. The biological material may be of a dry form or a wet solution. However, the present invention is clearly not limited to the materials listed herein.

Although it is preferred that a suspension of carrier particles and biological material or a suspension of biological material be used in accordance with the present invention, the present invention also contemplates other forms of biological material particles, i.e., in both dry form or suspended. For example, such biological material particles may include biological material which is freeze-dried or otherwise prepared as free particles or otherwise used as a particle for impact with target cells to penetrate such cells. Once the biological material particles have penetrated the target cells, such biological material particles or portions thereof would be expected to return to their natural state undamaged or otherwise contribute a desired biological activity within the target cell. For example, the biological material particles may return to their natural state by hydration, thawing, dissolving, etc.

The particle suspension from source **22** is provided to feeder **24** which controls the continuous flow of the source material to the electrospray dispensing device **12** when operable. The feeder **24** may be a liquid pump (e.g., a syringe pump, a gravity feed pump, a pressure regulated liquid reservoir, etc.), a mass flow controller, or any other flow control device suitable for feeding the source material to the dispensing device as would be known to one skilled in the art. The flow of a particle suspension, i.e., a solution, is atomized into microdroplets by the dispensing device **12**. Atomization may be provided by any known technique for producing microdroplets, which microdroplets preferably have a nominal diameter of about 10 nm or greater, more preferably about 20 nm to about 10 μm, and even more preferably about 30 nm to about 1 μm. Preferably, electrostatic atomization is used. However, other atomization devices (e.g., pressure regulated atomizers, ultrasonic nebulizers, hydraulic nozzles, etc.) may provide adequate atomization. As described in the papers entitled, "Electrospraying of Conducting Liquids for Dispersed Aerosol Generation in the 4 nm to 1.8 μm Diameter Range", by Chen et al., *J. Aerosol Sci*., Vol. 26, No. 6, pp. 963-977 (1995) and entitled "Experimental Investigation of Scaling Laws for Electrospraying: Dielectric Constant Effect, " by Chen et al., *Aerosol Science and Technology,* 27:367-380 (1997) which are hereby incorporated in their entirety by reference, microdroplets having nominal diameters in the range of about 10 mm to about 2 micron can be produced by electrospray. Various factors as described in such references affect the produced droplet size. For example, capillary size, liquid feed rate to the dispensing device, surrounding gas properties, etc. One skilled in the art will recognize that such factors and others may be modified to produce microdroplets of desired sizes.

By applying different electrical potential differences between the capillary tube electrode **18** and the second electrode **21**, different operating modes can be established. For example, a high positive voltage **20** is applied to capillary tube electrode **18** with the grounding of the electrode **21** to provide the spray **28** with a relatively high positive charge. For example, the high voltage source **20** may apply a high positive voltage preferably in the range of about 2000 volts to about 50,000 volts and more preferably 2000 volts to about 10,000 volts. The second electrode **21** in such a case may be provided to ground **38** or may have a negative voltage connected thereto. With relatively large potential differences being applied, as described in the above papers, pulsating modes or cone jet modes of operation are achieved. In a cone jet mode of operation, a cone shaped liquid meniscus is formed at the dispensing tip **23** whereas in the pulsating mode, the shape of the liquid meniscus alternates between a cone shape and a round shape. On the other hand, with relatively low electrical potential differences applied between the capillary tube electrode **18** and the second electrode **21**, dripping from the dispensing tip occurs.

One skilled in the art will recognize that a high positive voltage may be applied to electrode **21** with the tube electrode **18** grounded to provide a highly negative charge on the particles of the spray **28**. The only requirement necessary for the potential difference supplied between the capillary tube electrode **18** of the distributor head **19** and the second electrode **21** is that the electrical potential difference provides for a non-uniform electric field for establishment of a charged spray **28**. The charge on the particles of the spray **28** must be concentrated such that the space charge effect of the charged particles allows forcible contact with the target cells **40**, and preferably, allows for penetration of such target cells **40**.

It is noted that the particle velocity is established primarily by the space charge effect due to the concentrated charge on the particles of the spray. Only secondarily is the velocity of the particles provided by the attractive forces between the charged spray **28** and the second electrode **21**. It has been determined that for particles of relatively larger size, e.g., particles having nominal diameters less than about 0.5 microns, less than about 5 percent of the velocity is due to the electric field created by the applied voltage. Further, for particles of relatively smaller sizes, e.g. particles having nominal diameters of less than about 0.05 microns, less than 1 percent of the velocity is due to the electric field created by the applied voltage. Initially upon being dispensed from the dispensing tip **23**, the charged particle velocity is due to the electric field created by the applied voltage. However, such initial velocity is almost immediately overtaken by the tremendous velocity attainable due to the space charge effect of the charged particles. The second electrode **21** is primarily used for establishment of the charged spray forward of the dispensing tip **23**, and further is used for directing the particles of the spray and containment thereof.

Although various configurations for the dispensing device may be suitable, the dispensing device **12** preferably includes a capillary tube made of a suitable material, such as, for example, platinum, silica, etc. for providing the spray **28**. For example, the capillary tube may have an outer diameter in the preferred range of about 6 μm to about 2.5 mm and an inner diameter in the preferred range of about 6 μm to about 2 mm. Further, the dispensing device **12** may include a conductive or nonconductive casing concentric to the capillary tube, which is used to provide a sheath of gas, e.g., CO_2, SF_6, etc., around the capillary tube to increase the electrostatic breakdown voltage for the capillary tube, e.g., to prevent corona discharge. The use of such a sheath of gas is particularly beneficial when the spray is created using a high surface tension liquid, e.g., deionized water. Several detailed configurations for the dispensing device **12** are described in further detail below.

The desired velocity to which the particles of the spray **28** are accelerated depends upon various factors. For example, such factors include but are clearly not limited to the charge on the particles, whether a vacuum chamber is used, the size and density of the particles as well as the type of target cells **40** to be impacted. Preferably, the desired velocity is the minimum velocity necessary such that the particles can penetrate the cell membrane of the target cells **40**. The velocity necessary to penetrate such cells will be dependent upon the type of target cell which, for example, may include bacteria, single cell protozoa, plant pollen, plant protoplast, embryos, callus tissue, animal cells including animal progenitor cells (including, but not limited to pluripotent cells, stem cells, eggs, oocytes, embryotic cells), animal bone marrow cells and precursor cells, muscle or epidermal cells, epithelial cells, blood cells, isolated tissue explants, various other plant cells, or various other animal cells. The target cells may be part of a tissue, may be a monolayer of cells, a multilayer of cells, a suspension of cells, as well as being affixed to a surface or may take any other form as would be readily apparent to one skilled in the art.

With the preferred configurations as described herein, velocities in the range of about 30 m/sec to about 600 m/sec for particles having nominal diameters in the range of about 2 nm to about 1 μm are possible. The velocities on the higher end of the range are primarily due to small particle size, high particle charge, and/or reduced pressure. Further, particles can be generated utilizing such a configuration and delivered to the target surface at rates in the range of about 10^8 particles per second to about 10^{11} particles per second continuously. The particle generation rate may be increased by using multiple capillary tube electrodes. The preferred velocity, nominally in the range of about 150 m/sec to about 300 m/sec, is sufficient to penetrate but not cause damage to most types of target cells.

The spray of particles **28** established by electrospray dispensing device **12** when source **22** is a suspension including carrier particles and biological material is generally formed as previously described herein by dispensing microdroplets having the carrier particles and biological material suspended thereby. Thereafter, the microdroplets evaporate concentrating the charge of the microdroplets on carrier particles and biological material which typically becomes associated therewith. Likewise, the spray of particles **28** established by electrospray dispensing device **12** when source **22** is a suspension including bulk biological material is generally formed as previously described herein by dispensing microdroplets having biological material suspended thereby. Thereafter, evaporation of the microdroplets concentrates the charge of the microdroplets on the biological material. By controlling various parameters of the electrospray apparatus, the amount of biological material delivered for impact with the target cells **40** can be controlled. Further, the velocity of such particles may also be enhanced.

Several characteristics that can be controlled include microdroplet size, the concentration of biological materials, and carrier particle size of the particles suspended in the spray **28**. First, velocity of the particles may be enhanced by controlling particle size, i.e., carrier particle size. Smaller dimensional particles may enable such particles to have higher velocities due to space charge effect.

Further, by controlling the size of the sprayed microdroplets and the carrier particle size, the amount of biological material delivered can be controlled and higher velocities for the particles can be attained. First, the microdroplet nominal diameter can be controlled. For example, the microdroplet diameter may be controlled by controlling the capillary size, the liquid feed rate for suspensions, the electrical conductivity of the suspension, etc. The nominal diameter typically falls in the ranges as described previously herein.

With the use of carrier particles having smaller nominal diameters relative to the microdroplets, such as particles having a nominal diameter in the range of about 2 nm to about 1000 nm, or preferably in the range of about 10 nm to about 100 nm (or by increasing the size of the microdroplets relative to the particles), the amount of charge the carrier particles can carry is increased. In other words, by increasing the size differential between the microdroplets and the particles, upon evaporation the carrier particles (e.g., gold) can carry a charge much higher than the Raleigh limit for typical liquid suspensions. In this manner, the space charge effect provides for attainment of a higher velocity such that the particles can penetrate to different depths of the cell tissues. Further, by use of a vacuum chamber into which the particles are sprayed, increased velocities can be achieved.

Further, microdroplets having sizes slightly larger than carrier particles and/or biological material suspended thereby may be produced. This results in uniform size particles without agglomerates being formed. The effect of space charge repulsion of the unipolarly charged particles keep them separate and prevent particle agglomeration in the spray, as well as provide the particles with the velocity necessary for forcible contact with the target cells, preferably, for penetration of the target cells.

Further, by controlling the size of the microdroplet and the size of the carrier particles, one particle per microdroplet is attainable. With a controlled flow and known concentration of biological material utilized in association with the carrier particles, the amount of biological material in spray **28**, or delivered to the spray area, can be controlled and is reproducible, i.e., can be consistently repeated.

After the microdroplets of liquid suspending the particles and/or biological material, is dispensed, the solvent of the microdroplets begins to evaporate decreasing the size of the microdroplet. At the target cells **40**, typically only the carrier particle having associated biological material (or biological material alone in the case of a biological material suspension without carrier particles) remains for impact with the target cells **40**. The spray particle size can be made very small, as small as a few nanometers in diameter and still attain the necessary velocity under the effects of space charge. This makes it possible to deliver biological materials into smaller cells and tissues.

In addition to penetration of the cells as a result of the bombardment of the cells with material using the present invention, the electrospraying technique described herein may be used to produce liposome droplets encapsulating biological material, e.g., DNA. The liposome droplets can be directed by the electric field and distributed uniformly over target cells in manners similar to those described herein, e.g., movement of the target surface, movement of the distributor head, etc. As opposed to the penetration of the cells at impact, the liposomes encapsulating the biological material facilitate transfer of the material into the cells through fusion of the liposome with the cell membrane as is known to those skilled in the art. The liposome droplets may be of varying sizes, e.g., a nominal diameter of about 10 nm to about 10 μm. The electrospraying technique used to direct the liposomes onto the cells can be adjusted (e.g., distance of nozzle to target surface can be adjusted, electrical potential or strength of the field can be adjusted, etc.) to vary the velocity of the liposome droplets such that the liposome droplets land appropriately for the fusion mechanism to be accomplished.

One embodiment of an electrospraying apparatus **42** in accordance with the present invention is shown in FIG. **2**. Generally, the electrospray dispensing device **12** positioned for providing a spray **28** into a chamber **16** is substantially equivalent to that described generally with respect to FIG. **1**C as indicated by like reference numbers being used for like elements. However, in the embodiment of FIG. **2**, a target surface **14** having one or more target cells **40** placed thereon is positioned downstream from or forward of the dispensing device **12**.

The spray **28** has a first electrical charge concentrated on the particles as previously described herein upon evaporation of the microdroplets in the electric field created by the high voltage source **20** applied to the capillary tube electrode **18** and the grounding of target surface **14**. The capillary tube electrode **18** functions as the first electrode as described with reference to FIG. **1**B and the conductive target surface **14** functions as the second electrode described with reference to FIG. **1**B. With an electrical potential difference established between the capillary tube electrode **18** and target surface **14**, a nonuniform electric field is provided from the dispensing tip **23** to the target surface **14** to establish the spray of particles **28** forward of the dispensing tip **23**. In addition to creating the nonuniform electric field for establishing the spray of particles **28**, the conductive target surface **14** connected to ground **38** also provides for containment of the particles to a certain area to allow for forcible contact with the target cells **40**.

As shown in FIG. **2**, depending on the dispensing device used and other components of the apparatus, the particle suspension provided from source **22** may be from a pressurized source. Alternatively, a pressurized gas source **26** may be utilized in conjunction with feeder **24** or another portion of the dispensing device **12** so as to provide pressure to dispense the spray **28** into chamber **16**. For example, the source **22** may be pressurized in the range of a few tenths of an atmosphere to a few 100 atmospheres, or the pressurized gas source **26** utilized may be a gas such as, for example, carbon dioxide, ambient air, hydrogen, helium, nitrogen, and be at similar pressures.

The target surface **14** may be any suitable surface for placement of target cells. For example, the target surface may be an electrically conducting surface connected to an electrical ground or charge. Generally, the cells may be made conductive to the target surface by the natural moistness of the cells or made conductive by any other manner, e.g., coating, nutriant solutions. The target surface **14** is a substantially horizontal surface capable of supporting the target cells thereon. The electrical potential or ground may be applied to the target surface such that a portion or the entire surface attracts the spray, e.g., the particles are directed to particular areas of the target surface by the electrical field set up by the potential difference applied between the distributor head and the target surface. Being able to direct the spray to a particular portion of the target surface is beneficial in that overspray is avoided. In other words, the spray is attracted to a portion of the target surface having a voltage applied thereto, e.g., a portion insulated from the other portions, where target cells have been positioned. It is even possible to adjust the electrical field between the distributor head **19** and the target surface **14** such that the particles are directed from one position of the target surface to another, such as by switching mechanisms or alternative voltage sources applied thereto.

Preferably, the target surface **14** is moveable along the x, y, z axes as indicated generally with arrows **33** and **34**. The target surface **14** is provided with, and supported by, a movable positioning member (not shown) which enables it to move the target surface along such axes. For example, the target surface may be moved either closer to or farther away from the electrospray dispensing device **12**. The distance between the dispensing tip **23** of the distributor head **19** in the chamber **16** and the target surface **14** is preferably in the range of about 5 mm to about 3 cm depending on the desired electric field and spray area (D) desired. However, this distance may vary depending on the specific application, and it is apparent that the spray area D will have a lesser diameter as the dispensing tip **23** of the distributor head **19** and target surface **14** are moved closer to one another.

Since all the particles of the spray **28** carry the same polarity charge as they are dispensed into the chamber **16**, the particles tend to repel each other and avoid agglomeration. The electrospray technique can cover the spray area D uniformly. It should be noted that the repulsion of the same polarity charged particles will, at least in part, determine the spread of the spray **28** dispensed in the chamber **16**.

FIG. **3** diagrammatically shows the electrospraying apparatus **42** of FIG. **2** with the addition of an external electrostatic field **50** for further accelerating the charged particles of spray **28** dispensed into chamber **16**. The external electrostatic field **50** is provided using ring electrodes **52** having a negative high voltage supply **54** connected thereto and further connected to ground **56**. The field accelerates the charged particles dispensed into the chamber **16** through the ring electrodes. In such a manner, the particles are further accelerated towards grounded target surface **14**. The voltages applied to the ring electrodes may be in the preferable range of about 200 V to about 5 kV.

It should be readily apparent to one skilled in the art that the acceleration of the charged particles of the spray **28** by the external electric field **50** may be needed for accelerating the particles to a desired velocity necessary for penetrating certain target cells **40** on target surface **14**. However, in accordance with the present invention, such added acceleration is generally unnecessary as the space charge effect provides the necessary velocity for such penetration.

FIG. **4** diagrammatically illustrates the electrospraying apparatus **42** as shown in FIG. **2** but further includes a placement control member **70**. Placement control member **70** includes an opening **71** which allows a portion of the particles of spray **28** therethrough for impact with target cells **40**. In such a manner, the particular target cells **40** to be impacted can be controlled.

FIG. **5** is a diagrammatical illustration of an alternative electrospraying apparatus **100** which is similar to the electrospraying apparatus **42** of FIG. **2** but which includes several additional beneficial components. The electrospraying apparatus **100** includes electrospray dispensing device **112** substantially identical to that shown in FIG. **1** but which dispenses the spray **128** into a vacuum chamber **116** evacuated by vacuum pump **142**. The velocity of the particles of spray **128** due to the space charge effect is generally greater as the pressure in chamber **116** is decreased. Preferably, the pressure in the chamber is in the range of about 1 atmosphere to about 0.1 atmosphere.

The electrospray apparatus **100** further includes a target surface **114** whereon target cells **140** are placed and which is connected to ground **138**. The target surface is rotatable around axis **119** utilizing a motorized positioning member **115**. The target surface **114** can then be rotated and a more uniform distribution of the particles of spray **128** is delivered to the target cells **140**. Further, the dispensing device **112** is movable along the x, y, z axis as represented generally by arrows **131** and **132**. As such, particles can be delivered for impact with the entire area of the rotating surface **114**. For example, the dispensing device **112** can be positioned away from the axis **119** (e.g., half the distance between axis **119** and the edge of the rotatable target surface **114**). In this manner, the spray **128** can be uniformly distributed on the cells on the target surface **114** as the target surface rotates.

FIG. **6** diagrammatically illustrates an alternative electrospraying apparatus configuration **200**. The electrospraying apparatus **200** includes a conveyer system **260** which is positioned relative to dispensing device **212**. Dispensing device **212** is like an electrospray dispensing device described herein. The electrical potential difference for providing the spray from the dispensing device **212** is applied using voltage source **220** and grounding the conveyor system **260**. The conveyer system **260** includes target surface **262** which is moved by motorized element **264**. In such a manner, the continuous providing of spray **228** with the continuous movement of target surface **262** having target cells **240** placed thereon provides for a mass production system for transfer of biological material to target cells.

It will be readily apparent that the cells to be modified by the biological material may be in their natural state, e.g., in situ. Such cells may be treated while in the body of an animal, i.e., in vivo, or when such cells are removed from the body, i.e., ex vivo. For example, tissues which can be bombarded include human tissue or other animal tissues such as epidermal tissue, organ tissue, tumor tissue, plant tissue and like, while in the body or removed from the body. A portable electrospraying apparatus for gene therapy or other specialized in situ applications, e.g., immunization, may be used. For example, in such a portable configuration, the target cells to be impacted by the charged particles may be situ cells, as opposed to the cells being on a target surface. An illustrative portable electrospraying apparatus is generally described below with reference to FIG. **9** and will generally include elements or components similar in function to the configuration of FIG. **1**C.

It should be apparent to one skilled in the art that the various elements described in reference to FIGS. **1-6** may be combined in a variety of manners and each alternative configuration of the electrospraying apparatus described herein is for illustration purposes only. For example, the vacuum chamber **116** described with reference to FIG. **5** may be utilized with the electrospray apparatus of FIG. **4**, the placement member **70** described with reference to FIG. **4** may be utilized in the electrospray apparatus **42** of FIG. **2**, the conveyer system **260** as described with reference to FIG. **6** may be utilized with the external electric field **50** as described with reference to FIG. **3**, etc.

FIG. **7** is a more detailed diagram of one configuration of a portion **300** of the electrospraying apparatus shown generally in FIG. **2** including a dispensing device **314** according to the present invention. As shown in FIG. **7**, spray **328** is sprayed into a chamber **303** defined by a housing **302** having an axis **301** therethrough. The housing **302** includes a first end **304** and a second end **306** connected therebetween with a cylindrical wall about axis **301**. Preferably, the housing **302** is a vacuum chamber which can be evacuated as described further below. It will be recognized that various configurations may be selected for creating the housing **302** and that the present invention is not limited to any particular configurations. The housing **302** is generally formed of insulative materials. For example, the cylindrical wall enclosure **308** is preferably a plexiglass cylindrical wall for visibility while the first and second ends **304**, **306** may be formed of various insulative materials. First end **304** may also be formed of conductive portions to facilitate application of voltages or ground to the capillary tube **320**.

The second end **306** of the housing **302** includes an end element **311** connected to the cylindrical walls **308**. Positioned relative to an upper surface **370** of the end element **311** is a target platform **312** upon which target cells can be positioned. For example, a tube, dish, or any other structure may be positioned on the platform **312** which includes cells or the cells may be positioned on the platform **312** without any additional structure. Further, a rotatable micrometer adjustment mechanism **310** is provided through a lower surface **371** of the end element **311** for contact with platform **312** such that the height of the platform **312** can be varied, e.g., the distance between the target cells **340** and the dispensing tip **380** of the dispensing device **314** can be adjusted. The platform **312** is formed of a conductive material, e.g., stainless steel, and may function as the second electrode of the dispensing device **314** for establishing spray **328** from the dispensing tip **380** of the dispensing device **314**.

The first end **304** of the housing **302** includes a distributor head **316** extending therethrough having an axis that is coincident with axis **301** for use in establishing the spray **328** in the chamber **303** in combination with conductive platform **312**. The distributor head **316** includes a capillary tube **320** having an axis therethrough coincident with axis **301**. The capillary tube **320** includes a first end **330** sealingly positioned in aperture **385** of the first end **330** by conductive sealing element **337** at the upper surface **383** of the first end **304**. The capillary tube **320** further includes a second end **332** positioned for dispensing spray **328** as desired. The capillary tube **320** may be made of any suitable material, such as, for example, platinum, silica, stainless steel, etc. and may be of any suitable size. For example, the capillary tube may preferably have an outer diameter in the range of about 8 μm to about 2.5 mm, and an inner diameter in the preferred range of about 6 μm to about 2 mm. More preferably, the inner diameter of the capillary tube is in the range of about 10 μm to about 200 μm.

Further, the distributor head **316** includes a nozzle portion or casing **322** which as illustrated in FIG. **7** is an elongate substantially cylindrical metal casing concentric with the capillary tube **320**. However, the casing **322** can be conductive or nonconductive. Further, the casing **322** can take any configuration or shape which allows for the flow of a sheath gas about the capillary tube **320**. Together, in this particular embodiment, the capillary tube **320** and the casing **322** form the capillary tube electrode of the distributor head **316** for use in providing the spray **328** into the chamber in conjunction with the conductive platform **312**. The casing or nozzle portion **322** includes a first end portion **336** which tapers at section **335** thereof to a narrower second end portion **338**. The second end portion **338** extends from the tapered section **335** and is concentric with the second end **332** of the capillary tube **320**. The narrow end of the tapered section **335** extends a preferable distance of about 5 mm to about 5 cm from the lower surface **385** of the first end **304**. The outer diameter of the second end portion **338** is preferably in the range of about 2 mm to about 5 mm and the inner diameter of the second end portion **338** is preferably in the range of about 0.1 cm to about 0.2 cm. The second end **332** of the capillary tube **320** extends beyond the second end portion of the metal casing or nozzle portion **322** towards the target cells **340** by a distance of preferably about 2 mm to about 5 mm. The nozzle portion **322** is formed of any suitable metal or nonconductive material such as stainless steel, brass, alumina, or any other suitable conductive or nonconductive material. The nozzle portion **322** is spaced from the capillary tube **320** by spacers **326** or other spacing structures. For example, a metal casing **322** may be deformed at particular portions, such as pin points or depressions, to create a neck for centering the capillary tube **320** therein.

The capillary tube electrode may take one of many configurations. However, of primary importance is that the capillary tube electrode provide an electrode for creating the nonuniform electric field and provide at least a gas sheath about the capillary tube to avoid corona discharge if spraying high surface tension liquids, e.g., deionized water. For example, in an electrospraying apparatus wherein the spray is established in a chamber, the capillary tube electrode may just include a capillary tube itself, as opposed to requiring a casing such as metal casing **322** to provide an annular space for flow of the sheath gas. In such a configuration, the chamber may be flooded with the gas for preventing corona discharge. Further, when spraying liquids other than high surface tension liquids, the gas sheath may not be required.

A gas inlet **348** is provided in the first end **304** of housing **302** to allow for input of a stream of electro-negative gases, e.g., CO_2, SF_6, etc., to form a gas sheath about the capillary tube **320**. The inlet is configured for directing a stream of an electro-negative gas in an aperture **350** between the concentric capillary tube **320** and the nozzle portion **322**. This gas sheath allows the applied voltage to be raised to higher levels without corona discharge, e.g., the electrostatic breakdown voltage for the capillary tube electrode is increased. The entire portion of end **304** or portions thereof may be formed of conductive materials to facilitate application of a voltage or ground to the capillary tube electrode. For example, sealing elements **337** may be nonconductive, but is preferably conductive to facilitate application of a voltage or ground to capillary tube **320**.

The first end **304** further includes an exit port **354** for gases to exit the chamber **303**. For example, the exit port **354** may open into an annular chamber **389** defined in the first end **304** having a bottom face plate **390** having a series of holes for allowing flow from the chamber **303** out through the exit port **354**. A vacuum pump may be connected to the exit port **354** for evacuating the chamber **303** to a low pressure. For example, preferably, the pressure in the chamber is in the range of about 1 atmosphere to about 0.1 atmosphere. Further, instead of or in addition to providing the gas sheath between the capillary tube **320** and the nozzle portion **322**, the chamber **303** may be flooded with a gas through the exit port **354** to increase the electrostatic breakdown voltage for the capillary tube electrode.

In one embodiment, the chamber **303** is flooded with the gas through the exit port **354** and then a flow in the preferred range of about 5 cc/min to about 200 cc/min is continued through the exit port **354**. Any port to the chamber **303** may be used for exit of gas from the flooded chamber, e.g., such as a port that is available for sensing pressure (not shown) in the chamber. When the chamber **303** is flooded, the gas sheath between the capillary tube **320** and the nozzle portion **322** may not be necessary. As such, flooding of the chamber is an alternative to the use of such a gas sheath between the capillary tube **320** and the nozzle portion **322**.

To establish the spray **328** in the chamber **303**, biological material is provided, e.g., suspended in a solution, and received in the first end **330** of the capillary tube **320**. Preferably, the flow rate of the suspension may be in the range of about 0.01 μl/min to about 5 μl/min. Preferably, a relatively high voltage, for example, in the range of about 2000 volts to about 6000 volts, may be applied to the platform **312** relative to the capillary tube **320** which is electrically grounded (or vice versa) to establish the potential difference between the first and second electrode of the spraying apparatus. In this particular illustrative configuration, capillary tube **320**, metal casing **322**, and sealing element **337** are conductive. Spray **328** is established forward of the dispensing tip **380** of the second end **332** of the capillary tube **320** per a mode of operation as previously described. The potential difference between the electrodes establishes an electric field therebetween causing the formation of a smaller filament at the meniscus formed at the dispensing tip **380** while attracting the suspension downward toward the target cells.

FIG. **8** is a more detailed diagram of an alternate capillary electrode configuration **400** for the distributor head **316** of FIG. **7**. Like reference numbers are used in FIG. **8** for corresponding like elements of FIG. **7** to simplify description of the alternate capillary configuration **400**. Generally, the alternate capillary electrode configuration **400** is substituted for or replaces the single capillary tube **320** of the structure shown in FIG. **7**.

The capillary electrode configuration **400** includes a first capillary tube **412** having an axis coincident with axis **301** for receiving biological material from a source, e.g., a suspension of biological material. Further, a second capillary tube **414** is concentric with the first capillary tube **412**. An annular space **487** between the inner and outer capillaries **412**, **414** is used to direct a stream of electrolyte liquids of controlled conductivities to the dispensing tip **495** for use in establishing the spray forward thereof. The use of an electrolyte solution flowing to the dispensing tip **495** for establishing the spray of microdroplets therefrom, allows the suspension of biological material to be prepared with deionized water which has characteristics, e.g., pH, that do not disturb the biological material properties. With use of the electrolyte solution, sufficient charge is achieved on the microdroplets which thereafter concentrates on the particles of the spray to allow space charge effects of the particles to attain sufficient velocities for penetrating target cells. Without the second flow of electrolyte solution, an electrolyte solution may need to be added to the suspension to achieve such charge concentration. Further, the electrolyte solution characteristics, e.g., conductivity, can be changed to adjust the charge concentrated on the particles without the need to change the suspension characteristics. The stream of electrolyte liquids is directed in the annular space **487** such that it comes into contact with the suspension proximate the dispensing tip **495**.

In more detail, the housing portion **430** includes an aperture **483** extending from a first end **480** of the housing portion **430** to a second end **482** thereof. An inlet port **420** opens into the aperture **483**. The inlet port **420** receives a flow of electrolyte liquids **422** to be directed in the annular space **487** about the capillary tube **412**. The first capillary tube **412** has a first end **413** and a second end **415**. The capillary tube **412** is positioned in the aperture **483** of the housing portion **430** of generally T-shaped configuration. The first end **413** of the capillary tube **412** is sealed to housing **430** using conductive element **431** at the first end **480** of the housing portion **430**. The capillary tube **412** extends from the second end **482** of the housing portion **430** and with the second capillary tube **414** forms the annular space **487**.

The second capillary tube **414** includes a first end **490** and a second end **491**. The second capillary tube **414** is positioned so that it is concentric with the first capillary tube **412**. The first end **490** of the second capillary tube **412** is coupled to the second end **482** of the housing portion **430** using conductive element **432**. Further, the second end **491** of the second capillary tube **414** is held in place relative to the nozzle portion **322** by spacers **326**. The second capillary tube **414** extends beyond the first capillary tube **412** a predetermined distance in the direction of the target cells of preferably about 0.2 mm to about 1 mm. The portion of the second capillary tube **414** at the dispensing tip **495** which extends beyond the first capillary tube is tapered at a 60 degree to 75 degree angle for obtaining stable spray pattern and operation mode, i.e., consistent spraying patterns. Without the taper, intermittent operation may occur. Further, the second capillary tube **414** extends beyond the second end **338** of the nozzle portion **322** a predetermined distance (d**5**), preferably about 2 mm to about 5 mm. The first capillary tube **412** has preferable diameters like that of capillary tube **320** of FIG. **7**. The second capillary tube concentric with the first capillary tube has a preferable outer diameter of about 533.4 μm to about 546.1 μm and a preferable inner diameter of about 393.7 μm to about 431.8 μm. The gap d**6** at the tip of the second capillary tube **414** is preferably in the range of about 10 μm to about 80 μm. The other preferred configuration parameters are substantially equivalent to that described with reference to FIG. **7**.

In such a configuration, dual streams of liquids are provided for establishing a spray from dispensing tip **495** of the apparatus when a suspension of biological material or a suspension of carrier particles and biological material are used. This provides the benefits as previously described. Further, a gas sheath may also be provided through inlet port **348** as previously described with reference to FIG. **7**. Yet further, the first capillary tube **412** may extend beyond the end of the second capillary tube **414**, e.g., the dispensing tip is formed at the end of first capillary tube **412** which is closer to the target cells than the end of the second capillary tube **414**. In other words, the suspension may contact the electrolyte solution before exiting the dispensing tip **495** or the suspension may contact the electrolyte solution upon exiting the end of the first capillary tube **412**. Further, the second capillary tube may take various other configurations to form the space for providing the electrolyte solution to the dispensing tip, e.g., not necessarily a capillary tube structure.

The first or center capillary may be used to spray suspensions of biological material with or without the use of carrier particles. The rate of flow of such suspensions may vary. Preferably, the flow rate is about 0.01 μl/min to about 2.0 μl/min. The annular space between the inner **412** and outer **414** capillaries is used to direct the stream of electrolyte liquids of controlled conductivities. The rate of flow of such electrolyte liquids may vary. Preferably, the flow rate is about 0.1 to about 5 μl/min. For example, such electrolyte solutions may include deionized water with a trace of nitric acid, nutriant liquids used for growing cultured cells, or any other suitable component for biological material suspensions or target cells. The electrical conductivity of such electrolyte liquids is preferably in the range of about 60 $\mu\Omega^{-1}$/cm to about 80,000 $\mu\Omega^{-1}$/cm.

In addition to controlling conductivity and therefore the charge of the particles sprayed, the dual stream of liquids can further be used for other purposes. For example, the outer stream may be a suspension of liposomes that are sprayed with a suspension of other biological, e.g., DNA, provided through the center capillary. As such, the outer flow of the suspension includes an agent, e.g., liposomes, which is used to promote penetration of the target cells, e.g., dissolve the outer linings.

FIG. **9** shows an illustrative diagram of a dispensing device **500** for a compact pen-like electrospraying apparatus in accordance with the present invention that may be used for introduction of biological material into cells, such as in situ cells, e.g., human tissue or other animal tissues such as epidermal tissue, organ tissue, tumor tissue, plant tissue and the like. The dispensing device **500** includes a capillary tube **502** and a nozzle portion **504** configured substantially the same as described with reference to FIG. **7**. The apparatus further includes a gas sheath **509** provided between the capillary tube **502** and the nozzle portion **504**. The main difference between the apparatus as shown in FIG. **7** and that of FIG. **9** is that a ring electrode **530** used for establishing the spray at the dispensing tip **531** is positioned at the second end **533** of a cylindrical insulative jacket **514** concentric with and preferably in contact with the nozzle portion **504** along at least a portion of a first end **535** of the jacket **514**. With use of such a configuration, a chamber is eliminated.

EXAMPLE 1

Using an apparatus equivalent to that shown in and described with reference to FIG. **7** modified with the dual capillary tube distributor head **400** shown in and described with reference to FIG. **8**, biological material transfer was successfully accomplished. The apparatus used was configured with a center capillary tube **413** having an outer diameter of about 229 μm to about 241 μm and an inner diameter of about 89 μm to about 127 μm. The second capillary tube **414** concentric with the center capillary tube had an outer diameter of about 533 μm to about 546 μm and an inner diameter of about 394 μm to about 432 μm. The distance d**1** shown in FIG. **8** from the end of tapered section **335** to the end of the metal casing **322** is about 2 cm. The diameter d**2** of the first end **336** of the nozzle portion or metal casing **322** is about 0.5 cm. The outer diameter d**4** of the second end **338** of the nozzle portion **322** is about 1715 μm to about 1740 μm and an inner diameter d**3** of about 1333 μm μm to about 1410 μm. The distance d**5** from the tip of the second end **338** of the nozzle portion **322** to the tip of the end of the second capillary tube **414** is about 5 mm. The gap d**6** at the tip of the second capillary tube **414** is about 40 μm.

The dispensing device was constructed of various materials. Primarily, the conductive elements were constructed of stainless steel, the chamber wall was made of plexiglass, and the insulative parts such as portions of the ends **304** and **306** were made of a plastic, black delrin, material.

The biological material source was a suspension of plasmid and Au particles having 5 and 10 nanometer diameters (available from Sigma of St. Louis, Mo.). The plasmid was a commercially available plasmid including EGFP gene (Enhanced Greeen Fluorescent Protein from a Jelly Fish). The plasmid is available under the designation EGFP from Clontech of Palo Alto, Calif. The plasmid was resuspended for use at 0.05 μgrams/μliter in deionized water with a concentration of 0.01 percent Au particles.

The target cells were African Green Monkey fibroblast cells (COS-1) available from the American Type Culture Collection (Rockville, Md.) under the designation ATCC CRL-1650, Simian fibroblast like cells from kidney transformed with SV40 virus. The target cells were a monolayer at an estimated concentration of about 800 cells/cm^2. The target cells are in a Dulbecco's Modified Eagle Medium (DMEM-Hi) which includes 10 percent Fetal Calf Serum and 90 percent deionized water (available from Gibco/BRL of Rockville, Md.).

The electrospray was operated in a pulsating mode in a flooded chamber **302**. The chamber **302** was flooded using a 50 cc/min flow of CO_2 through port **354**. No gas sheath was provided about the second capillary tube **414**. A voltage of 4300 volts was applied to conductive element **312** as shown in FIG. **7**. The distance from the dispensing tip **495** of the second capillary tube **414** to the target cells **340** was about 2.5 cm. The cells were provided in a small well **396** (cut from a 12-well culture dish available from Corning of Cambridge, Mass.) formed of optically clear virgin polystyrene treated with optimal cell attachment and having a diameter of about 22 mm. The well **396** was placed on the platform **312** of the second end **306** of the housing **302**. Conductive wires **397** were provided from inside the well **396** to the conductive platform **312** to bleed off stray charge and to form the spray as well.

The sheath liquid provided in the annular space **487** between the first and second capillary tubes **412**, **414** was a 1 μl/min flow of deionized water plus a trace amount of nitric acid of a ratio of about (1:50) with an electrical conductivity of about 300 $\mu\Omega^{-1}$/cm. The suspension described above was provided by a syringe pump available under the designation of Harvard "33" Double syringe pump from Harvard Apparatus of Holliston, Mass. at a rate of 1.0 μl/min.

The cells were sprayed for about 2 minutes at a temperature of 20° C. and a pressure of 1 atmosphere. The well containing the target cells was placed in an incubator (available from NAPCO of Landrum, S.C.) for 1.5 days at a temperature of about 37° C., i.e., the time for cells to divide themselves and express fluorescence. A UV microscope available under the designation of Nikon Inverted Fluorescent Microscope from Fryer Co. of Minneapolis, Minn. was used to visually note the fluorescence. Approximately 40 percent to 60 percent of the cells fluoresced. As fluorescence was noted, introduction of biological material into the cell was successful.

EXAMPLE 2

The same setup of Example 1 was used. The only difference was that Au particles were not added to the suspension and the voltage applied to the element **312** was 5600 volts such that the dispensing device was operated in cone jet mode. Again, the Lw microscope was used to visually note the fluorescence. Approximately 40 percent to 60 percent of the cells fluoresced. As fluorescence was noted, introduction of biological material into the cell was successful.

All patents and references disclosed herein are incorporated by reference in their entirety, as if individually incorporated. Further, although the present invention has been described with particular reference to various embodiments thereof, variations and modifications of the present invention can be made within the contemplated scope of the following claims as is readily known to one skilled in the art.

What is claimed is:

1. A method of providing a plurality of particles for contact with one or more target objects, the method comprising:

providing one or more target objects;

providing an electrospray apparatus, wherein the electrospray apparatus comprises an inner opening and an outer opening concentric with the inner opening terminating at a dispensing tip of the electrospray apparatus;

providing a first flow of a liquid to the inner opening, wherein the first flow of the liquid comprises biological material;

providing a second flow of a liquid to the outer opening;

generating a spray of particles forward of the dispensing tip, wherein generating the spray of particles comprises dispensing a plurality of microdroplets having an electrical charge associated therewith from the dispensing tip by creating a cone jet from the first and second flow at the dispensing tip using a nonuniform electrical field between the dispensing tip and the one or more target objects, wherein the second flow of the liquid is used to adjust the conductivity of the first flow of the liquid comprising the biological material, and further wherein the plurality of particles having a nominal diameter of less than 1 micrometer are formed as the microdroplets evaporate; and contacting the one or more target objects with one or more of the plurality of particles using the nonuniform electrical field created between the dispensing tip and the one or more target objects.

2. The method of claim **1**, wherein the second flow of the liquid used to adjust the conductivity of the first flow of the liquid increases the charge concentration of the plurality of microdroplets upon generation from the dispensing tip.

3. The method of claim **1**, wherein the second flow of the liquid comprises an electrolyte liquid.

4. The method of claim **3**, wherein the electrolyte liquid comprises nitric acid.

5. The method of claim **1**, wherein the second flow of the liquid has an electrical conductivity less than 80,000 $\mu\Omega^{-1}$/cm.

6. The method of claim **1**, wherein the second flow of the liquid has an electrical conductivity greater than 60 $\mu\Omega^{-1}$/cm.

7. The method of claim **1**, wherein the electrospray apparatus comprises a compact pen configured electrospray apparatus comprising an electrode positioned at an end of an insulative structure of the electrospray apparatus and forward of the dispensing tip.

8. The method of claim **1**, wherein the electrospray apparatus further comprises a casing concentric with at least a portion of the first and second openings and defining a sheath opening terminating proximate the dispensing tip, wherein at least one of the first and second openings extend forward of the sheath opening in the direction of the one or more target objects, and further wherein the method comprises providing a gas to the sheath opening.

9. A method of providing a plurality of particles for contact with one or more target objects, the method comprising:

providing one or more target objects;

providing an electrospray apparatus, wherein the electrospray apparatus comprises:

a capillary tube electrode, wherein the capillary tube electrode comprises:

a first capillary tube having a first open end and a second open end, the capillary tube operatively connected to a biological material source to receive a first flow of a liquid comprising at least biological material at the first open end thereof, and an additional capillary tube concentric with at least a portion of the first capillary tube providing an annular space between the first capillary tube and the additional capillary tube for receiving a second flow of a liquid in the annular space, and

an electrode isolated from but positioned in proximity to the second open end of the first capillary tube, wherein a nonuniform electrical field is created between the capillary tube electrode and the electrode;

providing the first flow of the liquid to the fast open end of the first capillary tube, wherein the first flow of the liquid comprises biological material;

providing the second flow of the liquid to the annular space;

generating a spray of particles from the second end of the first capillary tube, wherein generating the spray of particles comprises dispensing a plurality of microdroplets having an electrical charge associated therewith from the second open end of the fast capillary tube by creating a cone-jet from the first and second flow at the second open end of the fast capillary tube using a nonuniform electrical field, wherein the second flow of the liquid is used to adjust the conductivity of the fast flow of the liquid comprising the biological material, and further wherein the plurality of particles having a nominal diameter of less than 1 micrometer are formed as the microdroplets evaporate; and

contacting the one or more target objects with one or more of the plurality of particles using the nonuniform electrical field.

10. The method of claim **9**, wherein the second flow of the liquid used to adjust the conductivity of the first flow of the liquid increases the charge concentration of the plurality of microdroplets upon generation from the second open end.

11. The method of claim **9**, wherein the second flow of the liquid comprises an electrolyte liquid.

12. The method of claim **11**, wherein the electrolyte liquid comprises nitric acid.

13. The method of claim **9**, wherein the second flow of the liquid has an electrical conductivity less than 80,000 $\mu\Omega^{-1}$/cm.

14. The method of claim **9**, wherein the second flow of the liquid has an electrical conductivity greater than 60 $\mu\Omega^{-1}$/cm.

15. The method of claim **9**, wherein the electrospray apparatus comprises a compact pen configured electrospray apparatus, wherein the electrode is positioned at an end of an insulative structure of the electrospray apparatus and forward of the second end of the first capillary tube.

16. The method of claim **9**, wherein the electrospray apparatus further comprises a casing concentric with at least a portion of the first capillary tube between the first and second open ends thereof and also the additional capillary tube to define an annular space between the casing and the additional capillary tube, the second open end of the first capillary tube extending beyond the casing a predetermined distance, and further wherein the method comprises providing a gas to the annular space between the casing and the additional capillary tube.

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